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# FORMULATION DEVELOPMENT AND INVITRO EVALUATION OF TOPICAL ETHOSOMAL DRUG DELIVERY SYSTEM OF KETOCANAZOLE

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**Abstract:**
*Ketoconazole is used in treating athlete's foot, jock itch, ringworm, Candidiasis and relieving the itching, scaling, burning, and discomfort due to those conditions. It may be used to treat yeast infections of the skin or scaly patches on the skin caused by fungus.[1] Ethosomes are capable of encapsulate high concentration of drug and delivers sufficient quantity of drug in small amount of dosage form. Ethosomal delivery of Ketoconazole may show greater penetration rate due to smaller average particle size than other vesicular systems. The main objective of the present study is to formulate and evaluate ethosome containing Ketoconazole for sustain drug delivery and to enhance the quick permeation of drug across skin.*

**Key words:** *ketoconazole, ethosomes, topical, drug release*

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## INTRODUCTION:

Optimization of drug delivery through human skin is important in modern therapy. Recently, the transdermal route vied with oral treatment as the most successful innovative research area in drug delivery[1].Transdermal delivery is an important delivery route that delivers precise amount of drug through the skin for systemic action (1,2).

Discovery of new medicinal agents and related innovation in drug delivery system has not only enabled the successful implementation of novel pharmaceutical, but also permitted the development of new medical treatment with existing drugs. Throughout the past two decades, the transdermal patches have become a proven technology holding the promise that new compound could be delivered in a safe and convenient way through the skin (2,3)

The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers are trying to understand the properties of vesicle structures for use in better drug delivery within their cavities that would allow tagging the vesicle for cell specificity (4,5,6). 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. One of the major advances in vesicle research was the finding a vesicle derivative, known as an ethosomes (7).

Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipids (Phosphotidyl choline; PC), ethanol at relatively high concentration and water. It was found that Ethosomes penetrate the skin and allow enhanced delivery of various compounds to the deep strata of the skin or to the systemic circulation (8,9).

Ethosomal gel formulation contains aqueous phase, oils, and emulsifiers for preparation of emulsion as a vehicle, gelling agents for gel preparation and penetration enhancers to increase the flux of drug through skin (10).

The objective of the present work is to formulate Ketaconozole ethosomal gel for topical drug delivery and to study the effect of concentration of carbopal in the formulation of gel.

## MATERIALS AND METHOD:

Ketaconozole was a kind gift sample from Natco pharma labs, Hyderabad, Cholesterol from Viratlab (Mumbai), Carbopol-934, Triethanol amine and Propylene glycol from Research lab fine chem. Industries (Mumbai) and all solvents used were analytical grade.

## EXPERIMENTAL

### Preparation of ketaconozole ethosomes

Preparation of Ketaconozole was followed by method suggested by Touitou et al., with little modification·100mg of Ketaconozole was dissolved in 6ml of water in a vessel and cholesterol was added to it with vigorous stirring. Propylene glycol was also added during stirring. The contents were heated to $30^0$c. In another closed vessel, soy lecithin was dissolved in ethanol with continuous stirring and heated to $30^0$ c. When both the solutions reached to same temperature slowly ethanol solution was added drop wise in the centre of vessel containing drug mixture. Then the stirring was continued for another 10min in a covered vessel. Water was added to adjust the volume up to 20 ml.

**Table-1: Composition of different Ethosomal gel formulations**

| Formulation (F) | Lecithin (%) | Propylene Glycol (%) | Ethanol (%) | Cholesterol (mg) | Drug (mg) | Water |
|---|---|---|---|---|---|---|
| $F_1$ | 2 | 10 | 20 | 0.05 | 100 | Q.s |
| $F_2$ | 3 | 10 | 20 | 0.05 | 100 | Q.s |
| $F_3$ | 4 | 10 | 20 | 0.05 | 100 | Q.s |
| **$F_4$** | **3** | **10** | **30** | **0.05** | **100** | **Q.s** |
| $F_5$ | 3 | 10 | 40 | 0.05 | 100 | Q.s |
| $F_6$ | 3 | 10 | 50 | 0.05 | 100 | Q.s |
| $F_7$ | - | 10 | 30 | 0.05 | 100 | Q.s |

* F7- Free suspension without vehicle forming agent.

**Preparation of Ketaconozole gel**

The best achieved vesicle suspension formula $EF_4$ was incorporated into Carbapol gel (1%, 1.5%, 2% w/w). The specified amount of Carbapol-934 powder was slowly added to pure water and kept at $100^0$c for 20min. Triethanolamine was added to it drop wise. Appropriate amount of ethosomal gel containing Ketaconozole was then incorporated into gel-base. Sufficient water was finally added with other formulation ingredients with continuous stirring until homogenous formulation was achieved ($G_1$, $G_2$ and $G_3$).

**CHARACTERIZATION OF ETHOSOMES**

**Size and shape analysis**

Microscopic analysis was performed to determine the average size of particles. A sample of gel was suitably diluted with distilled water in order to observe individual vesicle and a drop of diluted suspension was put on a glass slide covered with a cover slip. This was examined under microscope (magnification 15 × 45 X). The diameter of 150 vesicles was determined randomly using calibrated eyepiece micrometer with stage micrometer.

Sonication reduced the vesicular size. Since the vesicular size of these vesicles could not be analyzed using microscopic method at magnification 15×45X. Hence analysis of sonicated vesicles was done under a special microscope which was connected with software and photomicrographs were taken under 400 and 800 magnification.

**SCANNING ELECTRON MICROSCOPY (SEM)**

Determination of surface morphology (roundness, smoothness and formation of aggregates) of Ketaconozole gel with polymer was carried out by using scanning electron microscopy (SEM).

**Entrapment efficiency**

The entrapment efficiency of Ketaconozole drug into vesicle was determined by using ultracentrifugation. 10 ml ( Ketaconozole ) of each sample was vortexed for 2 cycles of 5 min with 2 minutes rest between the cycles. 1.5ml of each vortexed sample and fresh untreated Gel formulations were taken into different centrifugal tubes. These samples were centrifuged at 20,000 rpm for 3 hours. The supernatant layer was separated, diluted with water suitably and drug concentration was determined at 234 nm in both vortexed and unvortexed samples.The entrapment efficiency was calculated by considering the total amount of drug that was detected from supernatant of vortexed formulation and the amount of drug unentrapped and detected from supernatant of unvortexed formulation

**CHARACTERIZATION OF GEL**

**Surface morphology**

The surface morphology of the gel was determined by scanning electron microscope using gold sputter technique. The system was vacuum dried, coated with gold palladium, and then observed microscopically.

**Organoleptic Characters**

The formulations were tested for their psycho rheological properties like color, odor, texture, phase separation and feel upon application (grittiness, greasiness)

**Washability**

A small quantity of gel was applied on the skin. After washing with water, it was checked whether the gel was completely washable or not.

**Spreadability**

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

$$S=M/T$$

Where,

S = Spreadability in g/s

M = Mass in grams

T = Time in seconds

**pH measurement**

Solution was prepared by dissolving 1gm of Miconazol gel in 30ml of distilled water (pH 7). The pH of gel was determined by using digital pH meter. The measurement was done by bringing the probe of the pH meter in contact with the samples.

**Drug content and content uniformity**

1g of gel was dissolved in 100mlof phosphate buffer (pH 6.8) and kept for 48 hrs with constant stirring using magnetic stirrer. Then the solution was filtered and the absorbance was observed using U.V spectrophotometer at λmax i.e. 234nm. The measurements were made in triplicate.

**Drug release study from semi permeable membrane**

Semi permeable membrane mimicking skin was prepared by the use of egg membrane. For this an egg was taken and its contents were removed completely by making a small aperture at its tip. Then the egg was dropped in a beaker containing 100ml concentrated Hydrochloric acid (HCl). After about 15min the egg was turned to its other side. Leave the

egg in this position for about 30min. The conc. HCl dissolved egg shell leaving behind a semi permeable membrane. This membrane was collected and washed in distilled water thrice so that any remnants of egg or acid will be removed. This gives a semi permeable membrane which can be compared to skin. The membrane was mounted on a modified Franz diffusion cell in such a way that it remained in contact with the donor compartment.

The skin permeation of Ketaconozole from gel formulation was studied using Franz diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 2.4 cm and 20 ml respectively. The temperature was maintained at 37 ± 0.5°C [3]. The receptor compartment contained 20 ml of ph 6.8 phosphate buffer which was constantly stirred by magnetic stirrer at 100 rpm. The egg membrane was mounted between the donor and the receptor compartments. 1g of gel formulation was applied to the surface of membrane which was not in contact to the phosphate buffer. The content of diffusion cell was kept under constant stirring. 1 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analyzed by spectrometric method at 234 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh ph 6.8 buffer. Triplicate experiments were conducted for skin permeation study[6].

To analyze the in vitro release data, various kinetic models were used to describe the release kinetics. The zero order rate equation describes the systems where the drug release rate is independent of its concentration. The first order rate equation describes the release from system where release rate is concentration dependent.

## STABILITY STUDIES

Stability study was carried out for Ketaconozole Gel preparation at two different temperature i.e. refrigeration temperature (4 ± 2° C) and at room temperature (27 ± 2° C) for 8 weeks (as per ICH guidelines). The formulation was subjected to stability study and stored in borosilicate container to avoid any sort of interaction between the Gel preparation and glass of container, which may affect the observations.

Stability of drug and stability of vesicles are the major determinant for the stability of formulation. Studies were carried to evaluate total drug content at room temperature (27±2° C) and at refrigeration temperature (4±2° C). Samples were collected for every 2 weeks and absorbance was seen at 234nm in U.V spectrometer.

## RESULT AND DISCUSSION:

The gel formulations composed of phospholipids (lecithin, cholesterol), Ketaconozole and ethanol were prepared using the method detailed in last chapter titled materials and methods and also according to the literature with little modification in it. gel suspension was slight yellowish in color and hazy in appearance after sonication. Different characteristics of and the effect of sonication were further evaluated and results were reported under characterization.

### characterization of ketoconazole gel

Since the physical characterization is meant for physical integrity of the dosage form, the results were pooled at one place. Discussion on the results, described for gel formulation under the same heading.

### Size and shape analysis

Microscopic analysis was performed under different magnification to visualize the vesicular structure, lamellarity and to determine the size of gel preparations.

**Table 2: The size distribution of Ketaconozole gel formulations were as shown below**

| gel formulation | Average diameter (d avg) = $\frac{\Sigma nd}{\Sigma n}$ = |
|---|---|
| $F_1$ (2% Lecithin, 20% ethanol) | 4.44 µm |
| $F_2$ (3% Lecithin, 20% ethanol) | 5.106 µm |
| $F_3$ (4% Lecithin, 20% ethanol) | 5.483 µm |
| $F_4$ (3% Lecithin, 30% ethanol) | 5.302 µm |
| $F_5$ (3% Lecithin, 40% ethanol) | 4.122 µm |
| $F_6$ (3% Lecithin, 50% ethanol) | 3.907µm |

### Scanning electron microscope (SEM)

Microphotographs (figure 8-11) showed that size of the was reduced. The results of size and shape are consistent with the observations.

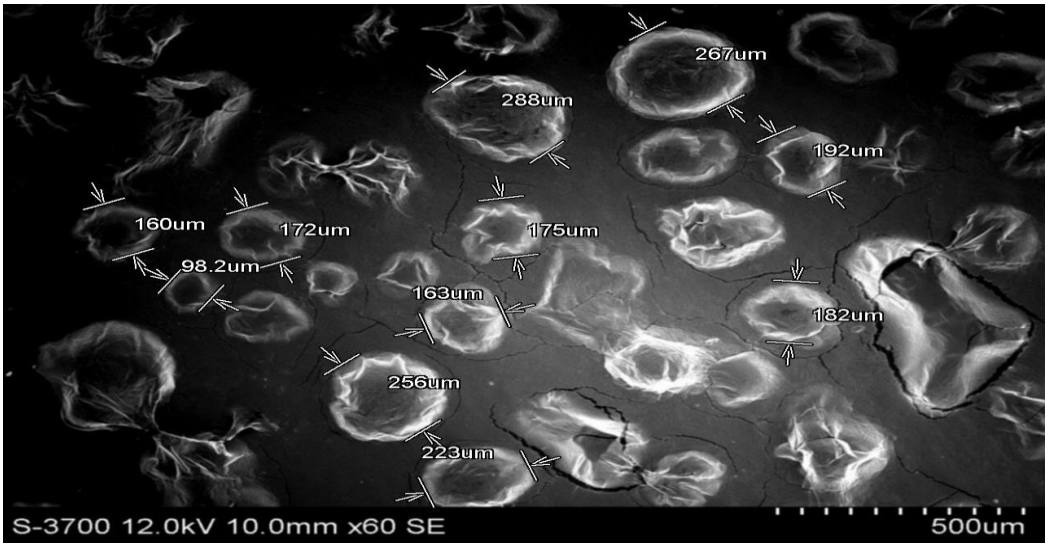


**Fig.1**

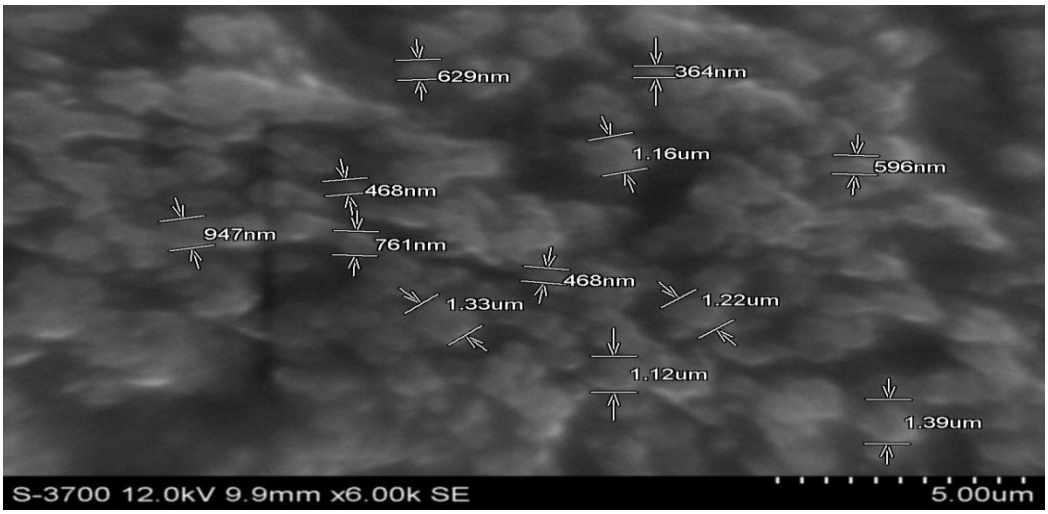


**Fig.2**

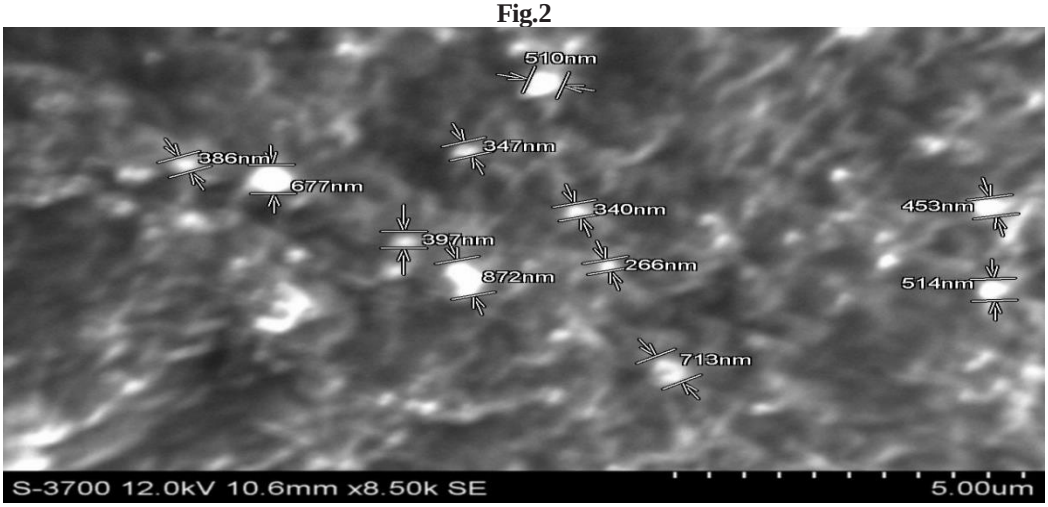


**Fig.3**

**Table-3: Drug entrapment efficiency of Ketaconozole Gel**

| Formulation code | Entrapment efficiency (%) | | | MEAN |
|---|---|---|---|---|
| F1 | 72.19 | 71.75 | 71.82 | 71.92 |
| F2 | 66.91 | 67.12 | 68.53 | 67.52 |
| F3 | 60.05 | 60.00 | 60.01 | 60.02 |
| **F4** | **79.91** | **79.62** | **79.33** | **79.62** |
| F5 | 58.01 | 55.96 | 54.96 | 56.31 |
| F6 | 39.39 | 42.32 | 42.76 | 41.49 |

The results obtained by vesicular size analysis showed concentration of ethanol effects vesicular size. The size of decreased as the concentration of ethanol increased with the largest vesicles size of 5.483 µm containing 20% ethanol and smallest 3.907 µm containing 50% ethanol. Results obtained in the present investigation are in conformity

**ENTRAPMENT EFFICIENCY**

Once the presence of bilayer vesicles was confirmed in the gel system, the ability of vesicles for entrapment of drug was investigated by ultra centrifugation. Ultra-centrifugation was the method used to separate the gel vesicles containing drug and un-entrapped or free drug, to find out the entrapment efficiency.

The maximum entrapment efficiency of gel vesicles as determined by ultracentrifugation was 79.62% for gel formulation containing 30% ethanol (EF4) which was almost double to the formulation containing 50% ethanol (EF6). As the ethanol concentration increased from 20% to 50% w/w, there was an increase in the entrapment efficiency and with further increase in the ethanol concentration (>30% w/w) the vesicle membrane became more permeable and that lead to decrease in the entrapment efficiency. Results of entrapment efficiency also suggest that 3% phospholipid concentration is optimum for entrapment efficiency. Any increase or decrease in concentration of phospholipid reduces the entrapment efficiency of vesicles. These result further supported the observation made by Jain NK et al.,[7]

Entrapment efficiency of gel formulations are significantly different and are reported in Table 3.

Increase in entrapment efficiency may be due to the possible reduction in vesicle size. There is a detrimental effect on the vesicles during ultra-centrifugation which are larger in size. Sonication gives more uniform lamellae with smaller vesicle and uniform size. Hence it may be the reason for higher vesicular stability and lesser vesicular disruption during ultra centrifugation.

***IN-VITRO* DRUG PERMEATION STUDIES**

The objectives in the development of in-vitro diffusion tests are to show the release rate and extent of drug from the dosage form. The in-vitro drug permeation study of Ketaconozole from gel formulation was studied using Franz diffusion cell and the method described in methodology chapter.

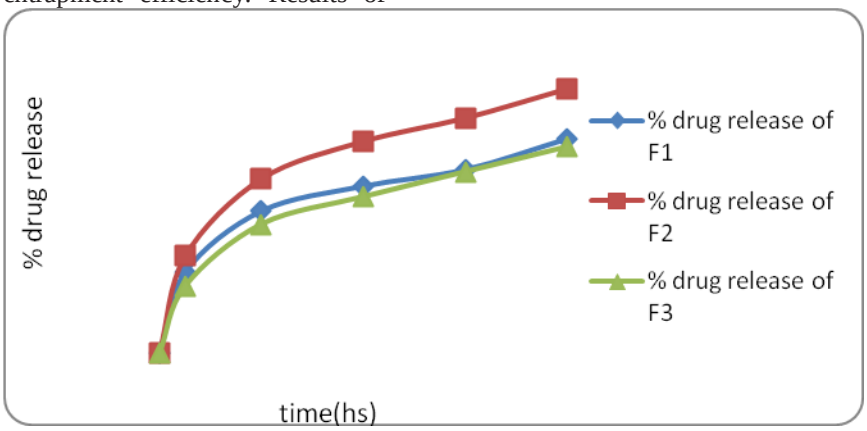


**Fig.4: In-vitro drug release studies of different gel formulations**

The release data was obtained for all the gel formulations. Spectrometric results were obtained and given consideration to sampling loss, to calculate actual cumulative drug diffused was calculated since the volume of receptor cell was only 20 ml . The obtained diffused amount of drug was extrapolated to diffusion by unit surface area of semi permeable membrane. These cumulative values were plotted as a function of time and steady state transdermal flux was calculated from the slop of linear portion.

The above figure is the graphical representation of the in-vitro drug release studies of different gel formulations from F1-F3 and showed decreased drug release. From the above the f3 formulation was selected as optimized formulation.

## CONCLUSION:

Ethosomal system is a highly efficient drug delivery system for topical drug delivery. Ketoconazole ethosomal gel can be effectively used for the management of topical infection.

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