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Research Article

# Comparative Evaluation of Sonicated and Un-Sonicated Ethosomes Containing Ketoconazole

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**ABSTRACT**

Aim of this work was to prepare and characterize sonicated and un-sonicated Ketoconazole encapsulated ethosomes as preferred formulation in the field of topical antifungal therapy. Drug encapsulated ethosomes were prepared using "Hot" method technique. Vesicles were evaluated for size, shape, entrapment efficiency, stability and *in-vitro* permeation using suitable analytical methods. Vesicle size of ethosomes containing 30% ethanol were found to be 1.158µm & 4.070µm with and without sonication. Microscopic observation showed that surface is smooth and vesicles are spherical in shape. Entrapment efficiency and stability of ethosomes was significantly improved with sonication and also with increasing the concentration of ethanol. *In-vitro* drug release of Ketoconazole was improved in case of ethosomes containing 30% ethanol with sonication.From these evaluations, the developed ethosomes of Ketoconazole demonstrated enhanced properties with increasing concentrations of ethanol as well as by subjecting vesicles for sonication.

**Keywords:** Ethosomes, Ketoconazole, Sonication, Ethanol, Delivery systems.

## INTRODUCTION

The hot, humid and tropical type of climate that prevails in most parts of world is one of the major cause for most of the diseases. This type of climate favours the growth of varieties of micro organisms, insects and other harmful disease causing pathogens. Thus, the proximity of occurrence of various diseases and infections in this country is also high. About 10% of annual funding for health research is spent on health problems that account for 90% of global disease burden. However only a fraction of new chemical entities are specifically indicated for topical diseases. Fungal, bacterial and viral infections have affected common heads greatly and through researches various treatment methods have been introduced. But it has been noticed that less care has been taken in studying the fungal infections and therefore the treatment for various dermatological infections due to fungus is minute. We feel that today this requires more attention and so the need for introducing more treatment techniques is the call of the hour[1].

Fungi usually make their homes in moist areas of the body where skin surfaces meet: between the toes, in the genital area, and under the breasts. Many fungi that infect the skin (dermatophytes) live only in the topmost layer of the epidermis (stratum corneum) and do not penetrate deeper. Obese people are more likely to get these infections because they have excessive skin folds. People with diabetes tend to be more susceptible to fungal infections as well[1]. Strangely, fungal infections on one part of the body can cause rashes on other parts of the body that are not infected. For example, a fungal infection on the foot may cause an itchy, bumpy rash on the fingers. These eruptions (dermatophytids or id reactions) are allergic

reactions to the fungus. They do not result from touching the infected area.

Fungi present an especially complex challenge to researchers. Research on fungal diseases focuses on three goals: providing better means of diagnosis, treatment, and prevention of the most important human fungal infections.Inspite of having various antifungal agents in different category of formulations still the disease seems to be a challenge for the society. But it is certain that, populations still do not consider the fungal infection as a major threat. The formulations presently available are ranging from tablets,capsules,ointments and creams, where the latest controlled delivery systems has been tried out in these formulations especially the liposomes and niosomes[2].These elite vesicular delivery systems have delivered their goals but still they do possess draw backs. Hence research is still progressing in the route and showed the possibility of newer vesicular system called ethosomes and their potential in antifungal therapy.

Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. These "soft vesicles" represents novel vesicular carrier for enhanced delivery to/through skin[3]. The size of Ethosomes vesicles can be modulated from tens of nanometers to microns**.** Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipids (Phosphotidyl choline; PC), ethanol at relatively high concentration and water. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin 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 improves drug distribution ability in stratum corneum lipids. 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. There are extensive work done by Touitou et.al in this field and the developed ethosomal systems found to be efficient in many aspects for most of the incorporated drugs[4].Ethosomes have shown their effectiveness to increase skin penetration of drugs to several folds than that of simple cream, liposomal earner and hydro-alcoholic solutions. Hence there is a need for preparation of Ketoconazole ethosomes for enhanced penetration through the skin, thereby reducing dose, minimizing frequency of administration and adverse effects, hence better patient compliance. A new possibility to treat deep dermal infections is by local application of antimycotic/ antifungal drug (Ketoconazole) in ethosomal carrier.

## MATERIALS AND METHODS

Ketoconazole was obtained as gift sample from Sharon bio-medicine limited, Raigad. Phospholipid, Ethanol and Potassium di-hydrogen phosphate purchased from Ozone chemicals, Mumbai. All other reagents and solvents were of analytical grade.

### Preparation un-sonicated ketoconazole ethosomes

Preparation of Ketoconazole ethosomes was followed by method suggested by Touitou et al., with little modification[3, 4].Ketoconazole 2% w/w solution was diluted with PBS pH 7.4. Phospholipids were dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel drug solution, ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach $40^0$ C. The organic phase is added to the aqueous one with stirring at 700 rpm. Alter mixing, stirring was continued for another 5 minutes. Temperature was maintained at 40°C for the entire process.Ethosomes prepared by the above procedure were subjected to sonication at 40° C using probe sonicator in 3 cycles of 5 min with 5-minute rest between the cycles[4].

### Size and shape evaluation[3-5]

Developed unsonicated ethosomes were evaluated for size and shape by using optical microscopy method. The average diameters of 150 vesicles were calculated. Sonicated vesicles were measured under a special microscope, which is connected with software and photomicrographs were taken under 400 and 800 magnification.

### Entrapment efficiency[4, 5]

The entrapment efficiency of Ketoconazole by ethosomal vesicles were determined by ultracentrifugation. 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 ethosomal 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 260 nm in both vortexed and unvortexed samples. The entrapment efficiency was calculated as follows

$$\text{Entrapment Efficiency} = \frac{T-C}{T} \times 100$$

'T' is total amount of drug that detected from supernatant of vortexed sample.
'C' is the amount of drug unentrapped and detected from supernatant of an unvortexed sample.

**Stability studies[6]**

Stability study was carried out for Ketoconazole ethosomal preparation at two different temperature i.e. refrigeration temperature (4 ± 2° C) & at room temperature (27 ± *2*° C) for 8 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any soil of interaction between the ethosomal preparation and glass of container, which may affect the observations. The ethosomal formulations were analyzed for any physical changes such as color and appearance and entrapment efficiency.

**in- vitro permeation studies[7]**

In-vitro permeation studies were carried out by using Franz diffusion cell, apparatus with a diameter of 25mm and a diffusional area of 4.90 $cm^2$. Regenerated cellulose acetate membrane (Thickness of 60-70μm and 0.50 μm pore size) was sandwiched between the lower cell reservoir and the glass cell top containing the sample and secured in place with a pinch clamp. The receiving compartment (volume 22ml) was filled with phosphate buffer pH 7.4 with 1% sodium lauryl sulphate.The system was maintained at 37 ± 0.5 $^{o}$ C. A Teflon coated magnetic bar continuously stirred the receiving medium to avoid diffusional layer effects. Sample was placed evenly on the surface of membrane in the donor compartment. 2 ml of receptor fluid was withdrawn from the receiving compartment at 0, 1,2,4,6,12,24,48 & 72 hours and replaced with 2 ml of fresh solution. Samples were analyzed spectrophotometrically for drug content at 260.2 nm.

**RESULTS AND DISCUSSIONS**

Ethosomal suspensions obtained by unsonicated and sonicated method were slight yellowish in colour and hazy in appearance. Different characteristics of ethosomes and the effect of sonication were evaluated and results are reported under the characterization.

Ketoconazole ethosomes 20% ethanol ($KET_1$) appeared as multilamellar vesicles. The lamellae of ethosome vesicles were evenly spaced to the core as this confirms the existence of vesicular structure at high concentration of ethanol. The size of $KET_1$ formulation was found to be within the range of 0 – 18.180 μm and the average diameter were found to be 5.325 μm. The size of Ketoconazole ethosomes containing 30% ethanol ($KET_2$) was found to be within the range of 0 – 16.750 μm and the average diameter was found to be 4.070 μm. The results (Table 1) obtained by vesicular size analysis showed concentration of ethanol affect vesicular size. The size of ethosomes prepared without sonication decreased as the concentration of ethanol increased with the largest vesicles size 5.325 μm containing 20% ethanol and smallest 4.070 μm containing 30% ethanol.

The formulations ($KET_1$ & $KET_2$) were subjected to sonication. Microphotograph showed that size of the ethosomes after sonication was reduced (Fig 1&2). The results of size and shape are consistent with the observations made by Jain N K et al[8]. Results (Table 1) showed the maximum vesicular size i.e. 1.515 μm for formulation containing 20% ethanol ($KET_l$) and minimum i.e. 1.158 μm for formulation containing 30% ethanol ($KET_2$ ). When the results of size distribution were compared, sonication was found to reduce the size of vesicles from 5.325 and 4.070 μm to 1.515 and 1.158 μm for ethosomes containing 20% and 30% ethanol respectively ($SKET_1$ & $SKET_2$). In average sonication bound to reduce the size to three or more times the initial.

The maximum entrapment efficiency of ethosomal vesicles as determined by ultracentrifugation was 68.33% for ethosomal formulation containing 30% ethanol ($KET_2$). As the ethanol concentration increased from 20% to 30% w/w, there was increase in the entrapment efficiency and as per Jain N K. et. al, further increase in the ethanol concentration (>40% w/w) the vesicle membrane may becomes more permeable that lead to decrease in the entrapment efficiency[8]. Results of entrapment efficiency also suggest that 2% phospholipids is the optimum concentration and beyond that entrapment efficiency may decrease.

Entrapment efficiency of sonicated vesicles found to be significantly higher than unsonicated (Table 2).The entrapment efficiency for sonicated ($SKET_1$ ) was found to be 57% and 78% for the $SKET_2$ .The increase in entrapment efficiency may be due to the possible reduction in vesicle size. The sonication gives the more uniform lamellae, smaller vesicle and uniform size and hence it may be the reason for higher vesicular stability and lesser vesicular disruption during ultracentrifugation.

Unsonicated ethosomal formulations were observed for any change in appearance or color for a period of 8 weeks. There was no change in appearance in ethosomal formulations throughout the period of study. Similarly the sonicated ethosomal formulations observed under magnified view and there was no indication of any increase in average

size or shape. The evaluation has confirmed the stability of formulation. The reason for such stability may be the presence of optimum level of phopholipid and ethanol in the formulations.

Since the stability of drug and stability of vesicles are the major determinant for the stability of formulations, studies were carried to evaluate total drug content and drug entrapment (Table 3) at room temperature (27±2° C) and refrigeration temperature (4±2° C). Loss in the percentage of drug content was not more than 5% .Highest drug loss was observed at room temperature after 8 weeks as compared to refrigeration temperature. Results also showed that there was no significant change in formulations & the drug content in different formulations, indicating the stability of drug even after 8 weeks in the formulation.

Entrapment efficiency is the integral part of stability of vesicles[9], hence selected to show the stability of prepared formulation. The data (Table 3) obtained indicate that formulations stored at refrigeration temperature and prepared by sonication method were found to show higher entrapment efficiency when compared with all other formulations after a period of 8 weeks.

*In-vitro* drug release studies have been extensively done for vesicles[10].From the formulations $KET_2$ & $SKET_2$ were subjected for in vitro permeation studies so that effect of sonication on release can be assessed. Percentage drug release for the $KET_2$ was found to be 0 for up to 2 hours and after 4 hours drug release has started with 2.51% then after 6 hours it was found to be 7.66% .The release was found to be sluggish at the initial stages where as later on at $72^{th}$ hour release was nearly 50%.But in case of $SKET_2$ the percentage drug release was nearly 69% at $72^{th}$ hour. The release of Ketoconazole was found to be more significant in case of sonicated version of formulation .At the end of the study, on $72^{th}$ hour the percentage release was found to be 50.32% and 69.2% respectively for $KET_2$ & $SKET_2$ (Table 4).This shows that sonication on ethosomes may improve the percentage drug release which is already there in ethosomes due to the presence of ethanol. Hence it may be beneficial if ethosomes were subjected for sonication process.

## CONCLUSION

Ethosomes- enhancing lipid carriers containing ethanol have been exhibiting high encapsulation efficiency for wide range of molecules and were able to deliver the molecules in most effective manner. This study identified that ethosomes properties such as stability, entrapment efficiency and in-vitro drug release profile may be improved by subjecting it to optimized sonication process. Even though sonication can enhance the properties of ethosomes, but concentration of ethanol can be considered as the most significant part of a best ethosomal formulation. Based on these findings further study can be recommended in the ethosomal vesicles to make it a best tool in novel vesicular drug delivery systems.

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**Table 1: Average size of prepared ethosomal vesicles**

| Ethanol Concentration %w/w | Average size of vesicles (μm) | |
|---|---|---|
| | Ethosomal preparation without sonication | Ethosomal preparation after sonication |
| 20 | 5.325 | 1.575 |
| 30 | 4.070 | 1.158 |

**Table 2: Entrapment efficiency for ethosomes**

| Ethanol concentration % w/w | Entrapment efficiency (%) | |
|---|---|---|
| | Ethosomal preparation without sonication | Ethosomes after sonication |
| 20 | 49 | 57 |
| 30 | 68 | 78 |

**Table 3: Percentage drug content after 8 week stability studies**

| Formulation code | Drug content in % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | | After 2 weeks | | After 4 weeks | | After 6 weeks | | After 8 weeks | |
| | 4±2 $^0$C | 27±2 $^0$C | 4±2 $^0$C | 27±2 $^0$C | 4±2 $^0$C | 27±2 $^0$C | 4±2 $^0$C | 27±2 $^0$C | 4±2 $^0$C | 27±2 $^0$C |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **$KET_1$** | 48 | 48 | 47.5 | 48 | 46 | 47 | 44 | 42 | 40 | 39 |
| **$KET_2$** | 69 | 69 | 69 | 69 | 68 | 67.5 | 62 | 63 | 60 | 59.5 |
| **$SKET_1$** | 55 | 55 | 55 | 54.5 | 53 | 53 | 50 | 50 | 49.5 | 48 |
| **$SKET_2$** | 75 | 75 | 75 | 75 | 74 | 73 | 72 | 72 | 71 | 70.5 |

**Table 4: In vitro release study data for selected ethosomal formulations**

| Time (In hours) | Percentage drug release | |
|---|---|---|
| | **$KET_2$** | **$SKET_2$** |
| 0 | 0 | 0 |
| 1 | 0 | 3.33 |
| 2 | 0 | 9.4 |
| 4 | 2.51 | 17.4 |
| 6 | 7.66 | 23.9 |
| 12 | 15.3 | 32.2 |
| 24 | 23.4 | 46.3 |
| 48 | 40.4 | 54.3 |
| 72 | 50.32 | 69.2 |

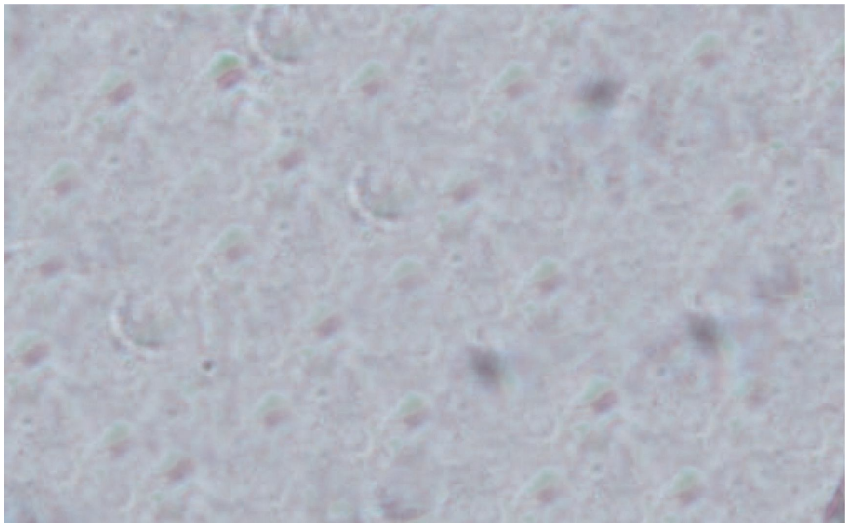

**Fig. 1: Photomicrograph for sonicated ethosomes $SKET_1$**

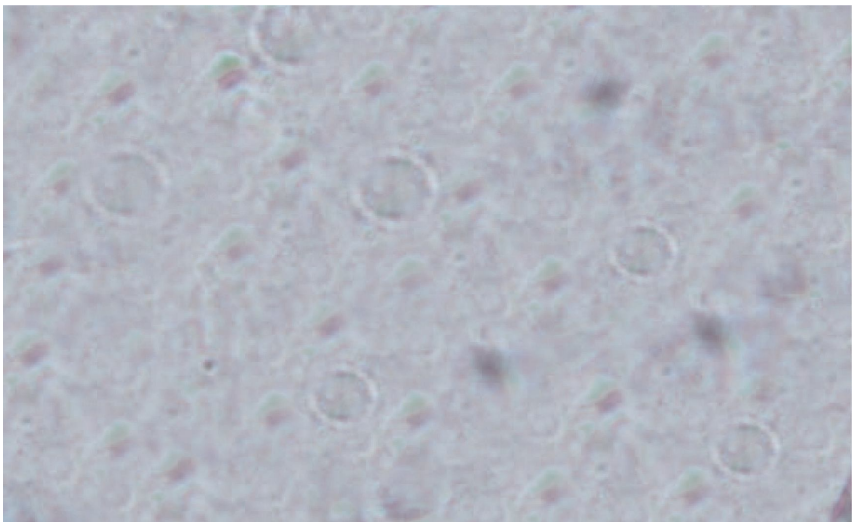

**Fig. 2: Photomicrograph for sonicated ethosomes $SKET_2$**

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