P-065 **Cubosomes: a tool for percutaneous administration of an antifungal agent**

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INTRODUCTION AND OBJECTIVES

Stratum corneum being the main barrier remains the outer most layer of the skin is mainly consisted of long chain ceramides, free fatty acids and cholesterol but the lipid phase behavior is different from that of other biological membranes. Crystalline phases are predominantly present but both the crystalline nature and the presence of a 13 nm lamellar phase poses a significant barrier to all the substances applied over skin (Bouwstra 2003). Miconazole nitrate (MCZ) is widely used in the treatment of fungal infection caused primarily by *Candida albicans*. One of the most common problems with this drug is that it does not reach into deeper layers of skin (Sparkes 2000). It is reported (Pershing 1994) that the inferior miconazole bioactivity was the result of decreased uptake into human stratum corneum. Therefore, to overcome all these limitations liquid crystalline nanoparticulate delivery system (cubosomes, hexosomes), can be used in drug delivery (Swarnakar 2007). Structure resemblance of cubosomes with membrane lipids and their tendency to fuse, may serve as an important mean to percutaneous delivery of bioactives at reduced dose. So, the objective of this work was to study the performance of cubosomes as percutaneous delivery systems for miconazole nitrate, chosen to increase its efficacy in treating deeper fungal infection (candidiasis) as well as restricting it to upper layers of skin.

MATERIALS AND METHODS

Glyceryl mono-oleate and poloxamer 407 were utilized in the preparation of cubosomes by utilizing hot-melt method as described by Esposito *et al.* (Esposito 2003) and characterized for particle size and distribution, particle isotropy, morphology and internal structure using dynamic light scattering method, cross polarizer microscopy, negative staining transmission electron microscopy (TEM) and small angle x-ray scattering (SAXS) respectively. Drug was incorporated in the lipophilic part.

Prepared formulation was studied for its transdermal potential across rat skin using modified Franz diffusion cell and tape stripping experiments were performed to ensure that drug loaded cubosomes remains in close vicinity of stratum corneum and epidermis. The extent of penetration has been studied by confocal laser scanning microscopy and to understand the probable mechanism of cubosomes penetration across stratum corneum FTIR has been employed as a tool.

RESULTS AND DISCUSSION

The entrapment efficiency of the system was 96 ± 1.2 % and the mean paticle diameter was 138 ± 2.1 nm. The developed system was characterized using cross polarizer microscopy revealed its isotropic nature. Further, cubic morphology of prepared formulation was clearly visible in TEM photomicrographs (Fig. 1).

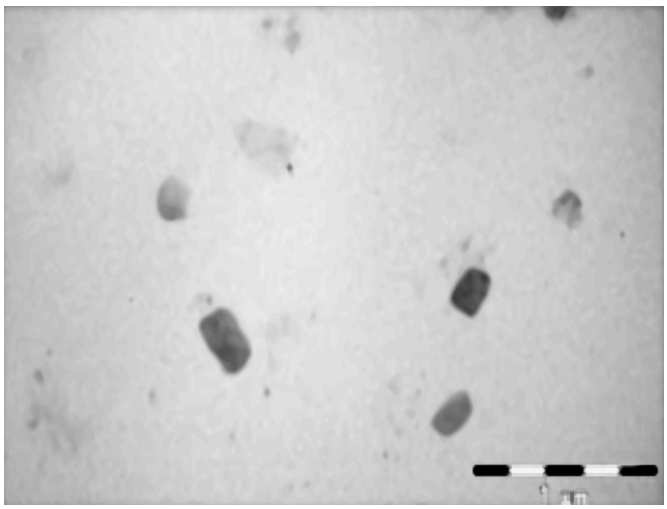

Figure 1: TEM of cubosomes

The SAXS analysis of plain (Fig. 2A) and drug loaded (Fig. 2B) cubosomes suggests Im3m cubic structure (three strong X-ray diffraction peaks with repeat spacing ratios of √2: √4: √6) and justifies that drug loading has not adversely affected the cubic geometry. About 11 fold increase in retention of miconazole nitrate in skin was achieved, which was confirmed by tape stripping experiments (Table 1).

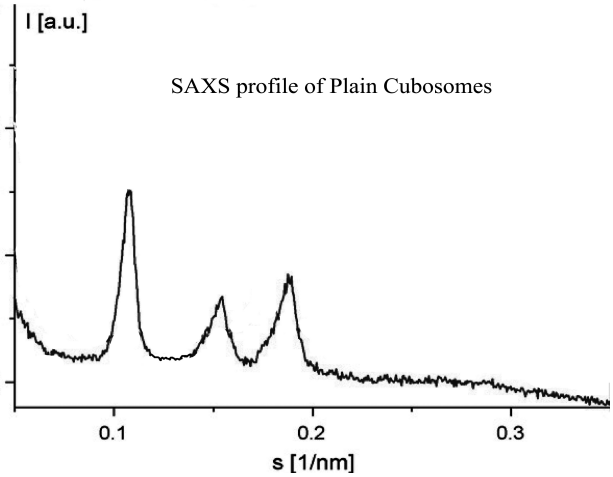

Figure 2A: SAXS profile of plain cubosomes

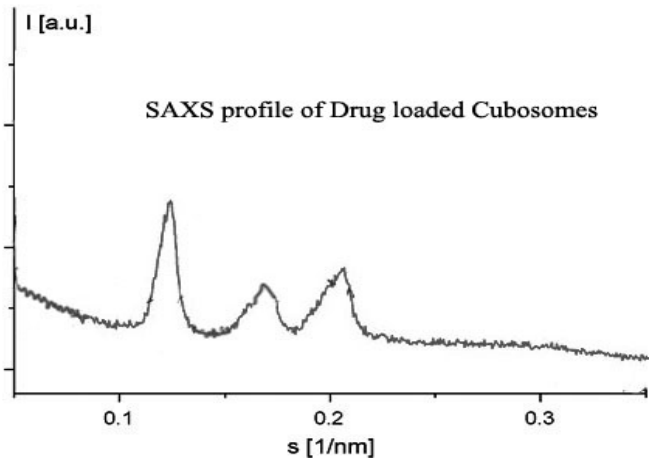

Figure 2B: SAXS profile of drug loaded cubosomes

Table 1: Tape Stripping Experiment

Formulation	% MCZ in skin after tape tripping	% MCZ in tape-strips (=% MCZ in stratum corneum)
MCZ Suspen-sion	1.6 ± 1.1	4.9 ± 0.97
MCZ in Gel form	2.1 ± 1.5	5.6 ± 1.85
MCZ + Gel + Cubosomes	25.6 ± 3.3	55.2 ± 4.2

Confocal laser scanning microscopy (Fig. 3) has been utilized to measure the extent of penetration and results suggest that cubosomes have penetrated upto a depth of 84 µm. The probable reason could be the structural resemblance of stratum corneum lipids to cubosomes and penetration enhancing properties of glyceryl monooleate (GMO). The FTIR analysis of cubosomes incubated with rat skin, in 3000-2800 cm^{-1} region (Fig. 5A) shows a shift in the C-H stretching peaks to higher wave number along with an increase in peak width and in the region of 1750-1500 cm^{-1} (Fig. 5B) show a decrease the strength of intermolecular hydrogen bond suggesting penetration enhancer effect of cubosomes by fluidizing the lipids present in stratum corneum.

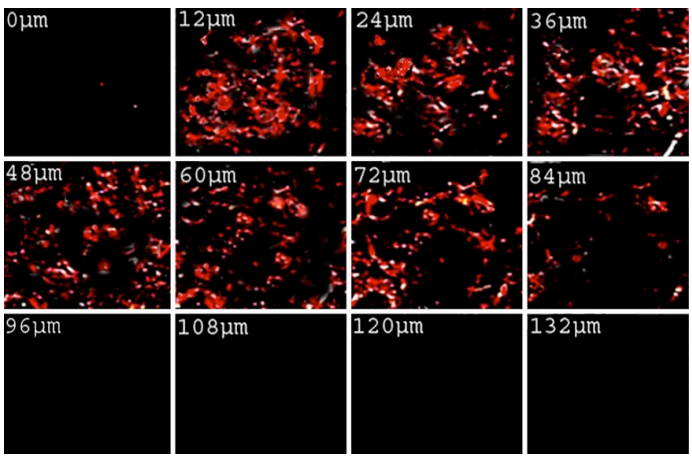

Figure 3: CLSM of rat skin

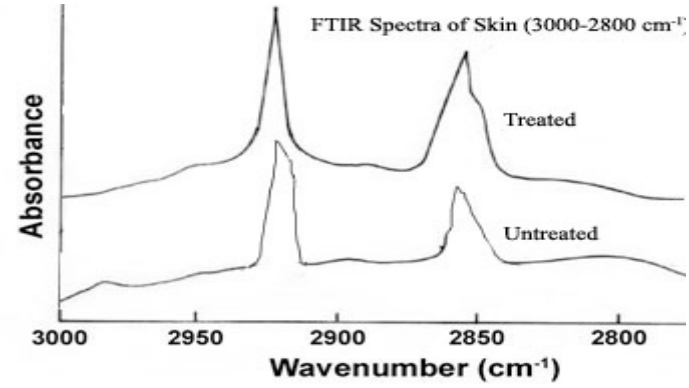

Figure 4A: FTIR spectra of skin (3000-2800 cm^{-1})

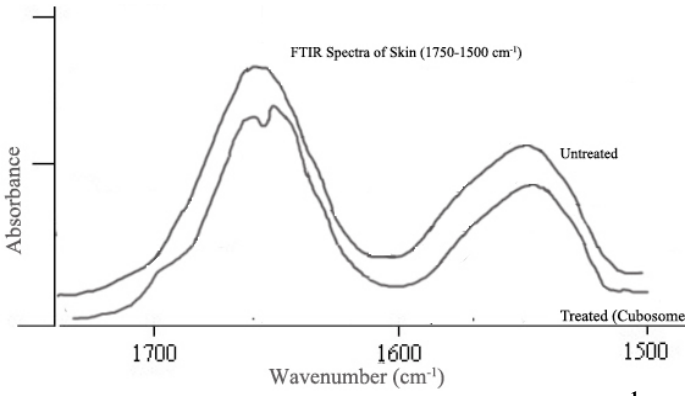

Figure 4B: FTIR spectra of skin (1750-1500 cm^{-1})

CONCLUSION

The present study suggests the potential of cubosomes as innovative drug delivery systems for percutaneous administration of antifungal drugs. The extent of skin retention and fluidization of lipids of stratum corneum as suggested herein may be employed as alternative approach beyond systemic administration to treat deeper fungal infections. Further exploration of the system alongwith *in-vivo* studies will generate a new era in field of percutaneous delivery.

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