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*Research Article*

# SPAN-60 NIOSOMAL ORAL SUSPENSION OF FLUCONAZOLE: FORMULATION AND *IN VITRO* EVALUATION

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## ABSTRACT

Niosomes have shown promise as cheap and chemically stable drug delivery systems. Niosomes have been prepared with different molar ratios of surfactant and cholesterol and their morphological properties have been determined by scanning electron microscopy. Different batches of Fluconazole niosomal preparations were prepared by changing the surfactant concentration but keeping the cholesterol concentration constant. The surfactant used was Span 60 and the five batches of niosomal preparations prepared were in the ratios 1:1:1, 1.5:1:1, 2:1:1, 2.5:1:1 and 3:1:1 (surfactant: cholesterol: drug). Furthermore, the release profile, entrapment efficiency, size distribution and stability of these niosomes under various temperatures were studied.

**Keywords:** Fluconazole, Niosomes, Ether Injection

## 1. INTRODUCTION

Current research and development strategy focus on development of drug delivery systems that make clinically established drugs do their therapeutic best rather than search for new drugs. The aim of any drug delivery system should always be to achieve maximum therapeutic response with minimum side effects.

Like phospholipids, the nonionic surfactants are able to form vesicular delivery systems called "niosomes" when dispersed in water. This is a rather recent concept with a few but very important investigations which show that this carrier system possesses great potential in delivery of drugs in biological system. They are known to be analogous to liposomes, and have been used in cosmetic formulations and experimentally as drug carriers[1, 2]. Niosomal vesicles can encapsulate both lipophilic and hydrophilic drugs and protect them against acidic and enzymatic effects in vivo[3].They offer several advantages over liposomes such as higher chemical stability, intrinsic skin penetration enhancing properties and lower costs [4]. However, there may be problems of physical instability in niosome dispersions during storage like vesicles aggregation, fusion, leaking or hydrolysis of encapsulated drugs, which might affect the shelf life of the dispersion [5].

Fluconazole, an antifungal drug, is available as capsules and suspensions. These formulations are used in the effective and safe treatment of oropharyngeal candidiasis. The topical effect of fluconazole oral suspension leads to an even faster resolution of symptoms. The aim of this study was to develop a niosomal drug delivery system for fluconazole , a triazole antifungal drug that acts by inhibition of the ergosterol component of the fungal cell membrane, because niosomal formulations have better permeability due to the presence of hydrophilic and lipophilic moieties. Hence a delivery system based on cholesterol and non ionic surfactant may have an advantage in the antifungal activity of fluconazole.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

Cholesterol, Span-60, sodium chloride, disodium hydrogen-phosphate, potassium dihydrogen-orthophosphate, sodium dihydrogen-phosphate and acetone were procured from Central Drug House Lab Reagents, Delhi, India. Dialysis membrane was purchased from Himedia Laboratories Pvt. Ltd; Mumbai, India and drug fluconazole was obtained as a gift sample from Idma Laboratories Limited, Chandigarh, India.

### *2.2. Methods*

#### *2.2.1. Preparation of niosomes*

Niosomes were prepared by slight modification of ether injection technique.[6]Niosomes were prepared using Span-60, cholesterol and fluconazole in the ratio (by weight) of 1:1:1; 1.5:1:1; 2:1:1; 2.5:1:1 and 3:1:1, respectively. For each ratio, non-ionic surfactant (Span-60) and cholesterol were weighed accurately and dissolved in 20 ml of chloroform. Fluconazole (20 mg) was then dissolved in this lipid solution. The resulting solution was taken in a syringe and injected slowly through a 16 gauge needle into 4 ml of aqueous phase (phosphate buffer pH 7.4) held in a beaker maintained at 60-65$^{o}$C and agitated slowly. As the lipid solution was injected slowly into the aqueous phase, vaporization of chloroform resulted in the formation of niosomes. These niosmes were filtered through 0.22 µm membrane filters after sonication for 20 minutes. The niosomal suspensions were labeled as F1-F5. The drug content in the niosomal vesicles were estimated by UV spectrophoto metrically at $\lambda_{max}$ 260 nm after disrupting the vesicles using 50% n-propanol.

*2.2.2. Microscopic evaluation of niosomes*

Small amounts of the formed niosomes were spread on a glass slide and examined for the vesicular structure and for the presence of insoluble drug crystals using ordinary light microscope with 10X magnification powers. The morphology of hydrated niosome dispersions prepared by conventional methods was determined using transmission electron microscopy. A drop of niosome dispersion was diluted 10-fold using deionized water. A drop of diluted niosome dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1 min to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion was removed by absorbing the drop with the corner of a piece of filter paper. After twice rinsing the grid (deionized water for 3–5 s) a drop of 2% aqueous solution of uranyl acetate was applied for 1 s. The remaining solution was removed by absorbing the liquid with the tip of a piece of filter paper and the sample was air dried. The sample was observed with a JEOL 100 CX transmission electron microscope at 80 KV.

*2.2.3. Determination of entrapment efficiency*

Niosomes containing fluconazole were separated from unentrapped drug by centrifugation. This purified niosomal dispersion was used for further studies. The niosomal dispersions were centrifuged at 9000 rpm for 30 minutes and the decanted fluid was separated from the sediment material i.e. the niosomes containing the entrapped drug [5].

An aliquot of freshly purified fluconazole niosomal dispersion after lysis with 50% n-propanol was analyzed spectrophotometrically for fluconazole concentration at $\lambda_{max}$ 260 nm to calculate the amount of entrapped fluconazole. The percentage of entrapped fluconazole was calculated by applying the following equation.

$$\% \text{ entrapment} = A_e x 100 / A_i$$

Where, $A_e$ is the amount of entrapped drug and $A_i$ is the initial amount drug in the lipid phase.

*2.2.4. Particle size determination*

Niosome size distribution was determined by dynamic light scattering (Zetasizer 3, Malvern, UK) at 25 °C. Samples were scattered (633 nm) at an angle of 90°. Each formulation was measured three times in triplicate during a period of 15 days.

*2.2.5. In vitro drug release*

Release of fluconazole from niosomes in vitro was performed according to Hu's method with minor modifications [5]. All the formulations were subjected to drug release studies [7]. A simple niosomal suspension was prepared (F6) for comparison. The dissolution cell consisted of a hollow glass cylinder (length 14.6 cm and internal diameter 2.5 cm) made up of Borosil glass. One end of the cylinder was covered with Himedia dialysis membrane (cut-off molecular weight: 12000-14000), which was previously soaked in warm water. The dissolution cell was placed in a 500 ml Borosil beaker that served as the receptor cell. The contents of the dissolution cell were agitated with the help of a glass stirrer. The receptor cell contained a magnetic bead and was rotated at a constant speed. The temperature in the dissolution and receptor cells was maintained at 37$^{o}$C, with the help of a thermostat. Five milliliters of each formulation was subjected to release studies. Phosphate buffer saline (100 ml) pH 7.4 was placed in the receptor cell. A 5 ml sample of each formulation was transferred to the dissolution cell. One milliliter samples were withdrawn from the receptor cell at specified time intervals of 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h. At each time immediately after the removal of the sample, the medium was compensated with fresh phosphate buffer saline (pH 7.4). The samples were analyzed for fluconazole content using a UV spectrophotometer (Shimadzu UV-160, Japan) at $\lambda_{max}$ 260 nm.

To investigate the possible mechanisms of fluconazole release from the prepared niosomes, the release data were analyzed mathematically according to the following models.

$Q = kt$

$\text{Log } Q = kt/2.303$

$Q = k\sqrt{t}$

Where, Q is the amount of drug released at a time (t) and k is the rate constant.

The cumulative percent release of fluconazole for all the batches was calculated and the data analyzed for release kinetics. Then the optimized batch was selected based on statistical inference and the optimized batch was compared with a niosomal oral suspension for its percent cumulative release.

*2.3 Stability studies*

The stability studies of the optimized niosomal formulation (F-2) were performed at different conditions of temperature and the effect on physical characteristics and drug content was noted. The niosomal dispersions (5.115 mg fluconazole /5ml) were kept in the air tight containers and stored at 2-8$^{o}$C and at room temperature (30 ± 2$^{o}$C) for 28 days and the 5.0 ml samples were withdrawn on different days (7, 14, 21 and 28). The samples were analyzed spectrophotometrically at $\lambda_{max}$ 260 nm after disrupting the vesicles with 50% n-propanol.

Table 1: Average particle size of fluconazole niosomes with varying concentrations of surfactant

| **Surfactant: Cholesterol: Drug** | **Mean Diameter (nm)** |
|---|---|
| 1:1:1 | 78.87±175 |
| 1.5:1:1 | 74.46±1.65 |
| 1:2:1 | 76.07±2.25 |
| 2.5:1:1 | 73.39±1.95 |
| 1:1:3 | 70.17±1.25 |

Table 2: Entrapment efficiency of niosomes with varying drug, surfactant, and cholesterol ratios

| Surfactant: Cholesterol: Drug | % Entarpment of Fluconazole |
|---|---|
| 1:01:01 | 92.71±0.43 |
| 1.5:1:1 | 92.27±0.52 |
| 2:01:01 | 93.18±0.37 |
| 2.5:1:1 | 91.36±0.98 |
| 3:01:01 | 91.81±0.73 |

Table 3: Release rate constants (k) and correlation coefficients (r) of niosomes

| Formulation | Zero Order Release Model | | First Order Release Model | | Higuchi Release Model | |
|---|---|---|---|---|---|---|
| | k (%$h^{-1}$) | r | k ($h^{-1}$) | r | k ($h^{-1/2}$) | r |
| F-1 | 0.964 | 0.801 | 0.008 | 0.781 | 11.600 | 0.865 |
| F-2 | 1.467 | 0.968 | 0.018 | 0.867 | 13.243 | 0.903 |
| F-3 | 1.250 | 0.905 | 0.014 | 0.815 | 11.748 | 0.853 |
| F-4 | 1.196 | 0.919 | 0.016 | 0.839 | 12.554 | 0.891 |
| F-5 | 1.019 | 0.880 | 0.007 | 0.789 | 11.477 | 0.837 |
| F-6 | 1.285 | 0.723 | 0.007 | 0.703 | 22.996 | 0.876 |

## 3. RESULTS AND DISCUSSION

### *3.1 Microscopic evaluation of niosomes*

Transmission electron microscopy (**Fig-1**) was used for microscopic evaluation of niosomal dispersions prepared by ether injection and it shows that niosomes formed by direct hydration are very heterogeneous and were both

unilamellar and multilamellar in their structures. Inverted microscopic evaluation (**Fig-2**) showed that most of the niosomes were mostly spherical in shape with a few being triangular, somewhat oval or slightly elongated and larger in size.

*3.2 Determination of particle size and entrapment efficiency*

Fluconazole niosomes prepared by ether injection technique employing varying ratios of surfactant (Span 60), cholesterol and drug were found to be spherical in shape. The average size of niosomes is reported in **Table-1**. The entrapment efficiency of drug was more than 91% with all the niosomal preparations as shown in **Table-2**. Cholesterol containing niosomes show higher entrapment because cholesterol alters the fluidity of chains in bilayer [8].

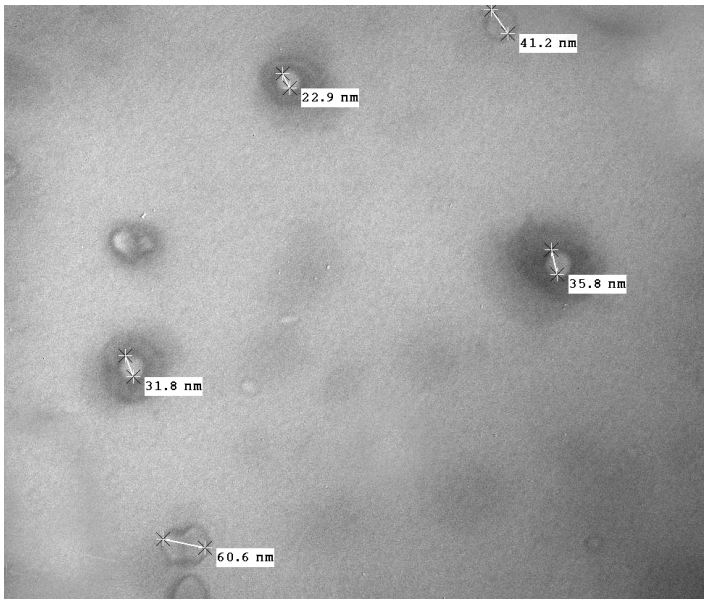


Fig.1: Transmission Electron Micrograph (TEM) of fluconazole the niosomes

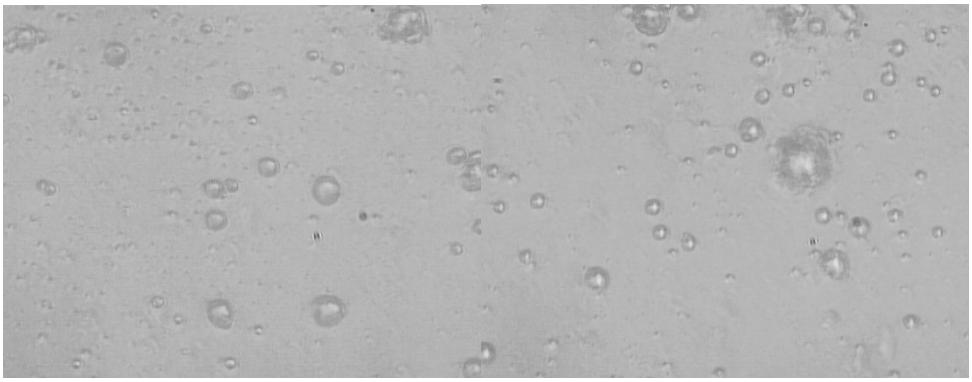

Fig.2: Inverted microscope photomicrographs of niosomes

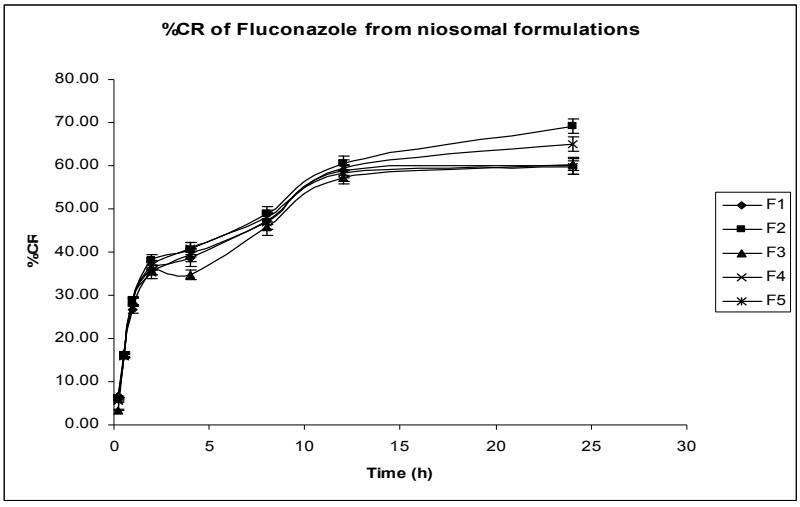


Fig.3: Cumulative fluconazole release of niosomal formulations

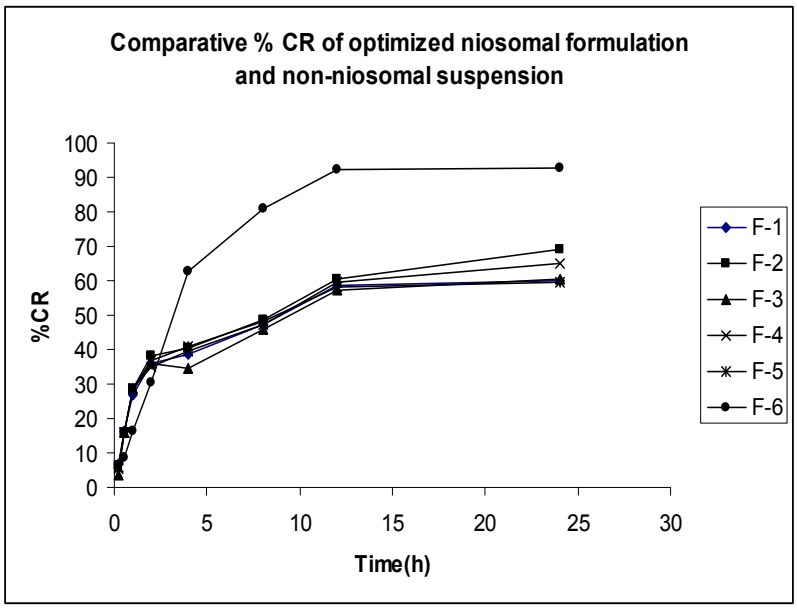


Fig.4: Comparative % CR of optimized niosomal formulation and non-niosomal suspension

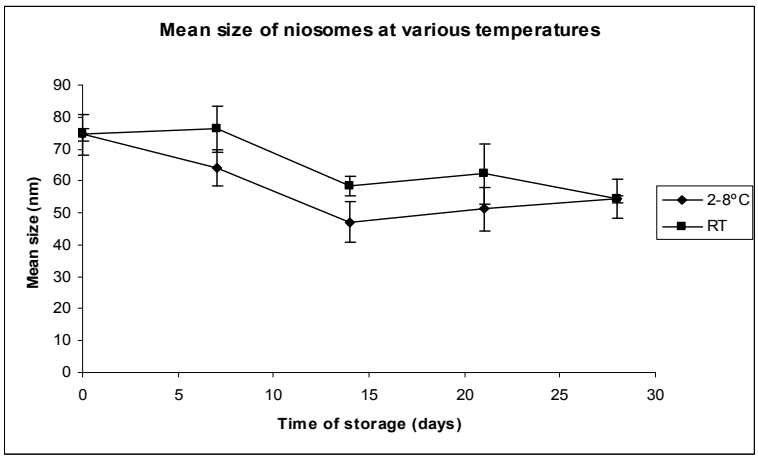


Fig.5: Mean size of niosomes at 2-8ºC and room temperature

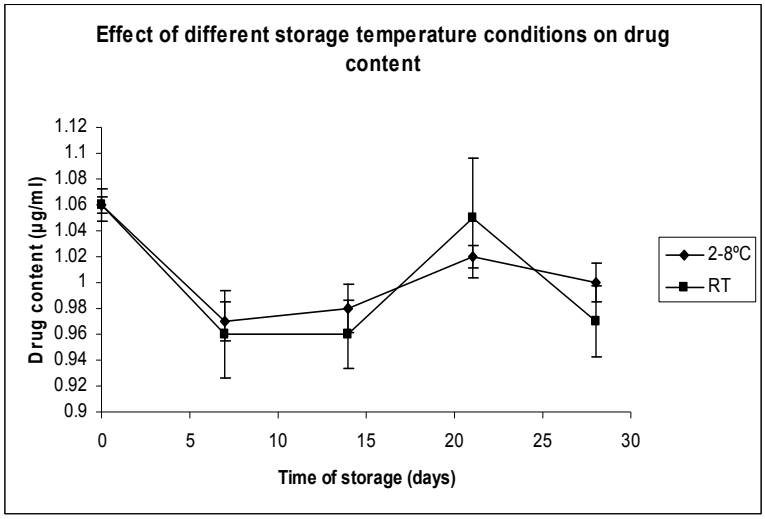


Fig.6: Effect of different storage temperature conditions on drug entrapment of oral niosomal suspension of fluconazole

*3.3 In vitro drug release*

To predict the release pattern of fluconazole from niosomal formulation batches (F-1 and F-5) correlation coefficient **(Table-3)** was calculated for zero order and first order kinetics. The release profiles of fluconazole from niosomes of different surfactant contents were apparently biphasic processes. The release pattern of F-2 is also the desired experimental release pattern expected i.e. sustained release matrix type. Therefore F-2 was an optimized batch and a comparative study of release pattern of F-2 with the prepared oral suspension showed significantly low cumulative release at every sampling interval. So it can be concluded that the fluconazole niosomal oral suspension is showing a slow and retarded release with no dose dumping in the dissolution media.

3.5 Stability Studies

Mean size determination and drug entrapped in niosomes at different temperature and stress are selected to establish the stability profile of the optimized formulation (F-2).

Mean particle size at different temperature was determined at weakly intervals for 28 days. Size fluctuations were observed though no aggregation of niosomes could be seen on storage. To determine stability, Fluconazole niosomal preparations were stored at 2-8ºC and at room temperature **(Fig-4)**. Although the mean size varied, there was no tendency towards increased aggregation and the storage temperature had little effect on particle size. Although mean size was seen to fluctuate, no substantial aggregation was seen. These experiments reveal that the Span 60 niosome formulations described herein are generally very stable. Storage at expensive sub-ambient temperatures offers no advantage and as Span 60 has a high phase transition temperature of 50$^{o}$C [9], room temperature would offer a suitable impermeable system for fluconazole entrapment.

Fluconazole niosomes were stored as the purified suspension with the unentrapped material removed by centrifugation, initially there was loss in fluconazole content within first seven days; there after the level of fluconazole fluctuate but not significantly (**Fig-5)**. Purified niosomes retain a great proportion of associated fluconazole on storage and it is difficult to explain initial loss seen as this in not simply an osmotic effect and it is unlikely the sufficient drug is associated loosely with the niosomal membrane. The drug content in niosomal formulation is higher at temperature 2-8ºC than at RT (30±2ºC)

## 4. CONCLUSIONS

Fluconazole was successfully encapsulated into niosomes by ether injection method, which showed that it was an appropriate alternative technique to load fluconazole into lipid vesicles. Niosomes offer advantages over other drug carriers with respect to biocompatibility, ease of preparation and their capacity to carry large

amount of drugs. The results reveal that niosomes are comparatively stable at lower temperatures. Thus on the basis of studies conducted we can state that niosomes possess great potential as drug delivery carriers and hence the formulated fluconazole niosomal suspension can be further evaluated for its *in vivo* performance.

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