Journal of Drug Delivery Science and Technology 39 (2017) 95–103

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

# Development and optimization of ketoconazole loaded nano-transfersomal gel for vaginal delivery using Box-Behnken design: *In vitro*, *ex vivo* characterization and antimicrobial evaluation

Shalu Singh [a], Devina Verma [a], Mohd. Aamir Mirza [b], Ayan Kumar Das [c], Mridu dudeja [c], Md. Khalid Anwer [d], Yasmin Sultana [a], Sushama Talegaonkar [a], Zeenat Iqbal [a, *]

[a] Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard University, New Delhi 110062, India
[b] New Zealand Fulvic Limited, Mount Mounganui, Tauranga 3116, New Zealand
[c] Department of Microbiology, Hamdard Institute of Medical & Scientific Research, Jamia Hamdard, New Delhi 110062, India
[d] Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-kharj 11942, Saudi Arabia

ARTICLE INFO

*Article history:*
Received 2 January 2017
Received in revised form
26 February 2017
Accepted 8 March 2017
Available online 11 March 2017

*Keywords:*
Ketoconazole
Transfersome
Nanogel
Permeation
Antimicrobial studies

ABSTRACT

In the present study, Ketoconazole loaded transfersomal formulation was developed using 3-factor, 3-level Box-Behnken design to find out the best formulation. Optimized transfersomal formulation was prepared by solvent evaporation method and evaluated *in vitro* for vesicle size (126.9 ± 5.45 nm) and entrapment efficiency (82.6%). Transfersomal gel were developed by incorporation of optimized transfersomal formulation into 1% carbopol gel base and characterized by physical evaluation and rheological studies. The cumulative release of drug were shown at 72 h approximately 74% and 97% of ketoconazole from suspension and transfersomal gels respectively. The flux for transfersomal gel was found to be about 3 times that of drug suspension gel. Results of the histopathological studies of gel treated skin indicated negligible sign of toxicity and irritation. The prepared transfersomal gel showed antimicrobial activity against Candida albicans with MIC 4.57–4.6 μg/mL and better zone of inhibition as compared to reference standard. The developed gel showed promising antimicrobial activity. These overall findings suggested that transfersomal gel holds an excellent potential for ketoconazole delivery.



## 1. Introduction

Ketoconazole, an anatifungal drug, is used to treat serious fungal or yeast infections, such as candidiasis, blastomycosis, coccidioidomycosis, histoplasmosis, chromoblastomycosis. This medicine works by killing the fungus or yeast, or preventing its growth [1–3]. Vulvovaginal candidiasis (VVC) is a common gynecological pathology occurring when there is a yeast infection that affects nearly 75% of all adult women [4,5]. Classically, VVC is described by a white “cheese” release alongside vulva and vaginal irritation. The most widely recognized reason for contagious contaminations is Candida species, prompting a scope of intrusively life-threatening to non-life-unthreatening mucocutaneous disease. Candida albicans is a demographic yeast that colonizes the skin, reproductive and the gastrointestinal tract [6]. The pathogenesis and prognosis of candidial contaminations are influenced by the host immune status furthermore vary enormously as indicated by infection presentations. Subsequently, diagnosis, managment, and treatment choice changes and ought to be considered in the general setting of the affected human host [7]. The treatment methods of Vaginal Candiasis primarily include topical treatment and long term treatment (3–5 months) may be most effective. The long term use of drug increases the chances of missing the dose and increasing the side effects. Therefore, it is better to develop a formulation which reduces the frequency of administration and which retains for long period of time [8].

Transferosomes are novel ultra deformable vesicular carrier system primarily composed of phospholipid, surfactant, and water. Because of their self-optimized and ultra-flexible membrane properties, they can deliver the drug into the vagina through the vaginal mucosa [9]. The vesicular transfersomes are more elastic than the conventional liposomes because of the presence of the

* Corresponding author. Nanoformulation Research Lab, Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard University, New Delhi 110062, India.
*E-mail address:* ziqbaljh@yahoo.co.in (Z. Iqbal).

http://dx.doi.org/10.1016/j.jddst.2017.03.007
1773-2247/

edge activators in the vesicular membrane and are thus well suited for delivering drugs through the vaginal mucosa as compared to the rigid liposomes [10,11]. This enhanced penetration of Transferosomes is associated by its ability to squeezing themselves along the intracellular sealing lipid of the stratum corneum. The extremely high flexibility of their membrane permits transfersomes to squeeze themselves even through pores much smaller than their own diameter. This is due to high flexibility of the transfersome membrane and is achieved by judiciously combining at least two lipophilic/amphiphilic components (phospholipids plus bio surfactant) with sufficiently different packing characteristics into single bilayer. Transfersomes has the potential of overcoming the skin barrier and have been reported to enhance the permeability of drug [12,13].

## 2. Material and method

### 2.1. Materials

Different phospholipids phospholipon 90 G and Lipoid S100 were obtained from Lipoid® GmbH (Germany). Tween 80 was purchased from Thomas Bakers (Mumbai, India). Ethanol was purchased from Jiangsu Huaxi International Trade, Co. Ltd., China. Ketoconazole was obtained as a generous gift from Sigma Aldrich (Germany). Water and other solvents used in the HPLC analytical method were of HPLC grade. All other reagents were of analytical grade and were purchased from Merck, Mumbai, India.

### 2.2. Experimental design for optimization of transfersomes using factorial design

A 3 factor, 3-level factorial design was used to explore the quadratic response surfaces and for constructing second for second order polynomial models using Design-Expert (Version 10, Stat-Ease Inc., Minneapolis, MN). A design matrix comprising of 17 experimental runs constructed, for which the non-liner computer generated quadratic model is defined as: $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12} \times 1 \times_2 + b_{13} \times 1 \times_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3$, where Y is the measured response associated with each factor level combination; $b_0$ is constant; $b_1$, $b_2$, $b_3$ are linear coefficients, $b_{12}$, $b_{13}$, $b_{33}$, are interaction coefficients between the three factors, $b_{11}$, $b_{22}$, $b_{33}$, are quadratic coefficients computed from the observed experimental values of Y from experimental runs; and $X_1$, $X_2$ and $X_i^2$ (i = 1, 2 or 3) represent the interaction and quadratic terms, respectively. The independent variables were selected as amount of Lipoid S 100 ($X_1$), Tween 80 ($X_2$) and ethanol ($X_3$). The dependent variables were particle size ($Y_1$), entrapment efficiency ($Y_2$), and poly dispersive index (PDI) ($Y_3$) with constraints applied on the formulation of transfersomes. The concentration range of independent variables under study is mentioned in the Table 1 along with their low and high levels.

### 2.3. Development of optimized transfersomes

Transfersomes were prepared by conventional solvent evaporation method. Optimized batch of transfersomes was prepared using surfactant, phospholipids and drug. Accurately weighed ketoconazole was dissolved in ethanolic solution of Lipoid S 100 in a beakeras organic phase. This organic phase were added slowly to aqueous phase containing surfactant dropwise with the help of syringe on continuous stirring and is kept for almost 6–7 h for solvent to evaporate. Then this mixture is sonicated for 2–3mins to obtain nano transfersomes particles [14].

### 2.4. Characterization of optimized ketoconazole transfersomal formulation

The *in vitro* behavior of the prepared formulation would be depicted through its characterization.

#### 2.4.1. Scanning Electron Microscopy (SEM)

The surface morphology of optimized transfersome was examined by SEM. 1–2 drops of vesicular dispersion was mounted on a glass and paste over grid by using double-sided carbon adhesive tape and sputter-coated with conductive gold-palladium. A round coverslip was gently placed over the stub to enable uniform conductivity and a silver paint lining was applied to the edges of the coverslip to fill the narrow spacing between the stub and coverslip. They were viewed with an EVO LS 10 (Carl Zeiss, Brighton, Germany) scanning electron microscope operating at an accelerating voltage of 200 kV under high vaccum. The particles were examined for surface characteristics like shape, size, pores, pits and presence of aggregation [15].

#### 2.4.2. Transmission Electron Microscopy (TEM)

Morphology and structure of the transfersome was studied using TEM (Morgagni 268 D FEI Company 155, Netherland). The samples were treated on copper grids (Polysciences, Warrington, PA, USA) without salt of heavy metal viz. 1% phosphotungustic acid for negative staining, followed by sample drying. They were then analyzed by TEM at an accelerating voltage of 200 kV and data acquisition was done on the AMT Image Engine [16].

#### 2.4.3. Vesicle size determination

The vesicles size and size distribution were determined by Dynamic Light Scattering (DLS) technique using a computerized inspection system (Malvern Zetasizer, Nano-ZS, Malvern, U.K.) with DTS (nano) software® [16]. Optimized vesicular suspension was diluted with distilled water and placed in quartz cuvette and then subjected to size analysis and the measurements were conducted in triplicate.

### 2.5. Determination of percent entrapment efficiency (PDE)

Entrapped transfersomal vesicle were separated from untrapped ketoconazole by centrifugation at 10000 rpm for 45 min. The concentration of ketoconazole in supernatant were measured by UV spectrophotometer. Percent drug entrapment was calculated by using following equation [1]:

$$PDE = W - w/W * 100$$

where W = theoretical amount of Ketoconazole; w = observed amount of Ketoconazole in supernatant.

**Table 1**
Considered variables and responses along with their levels and constraints.

| Factor | Levels | |
|---|---|---|
| Independent variables | Low | High |
| $X_1$ = Lipoid S 100 (mg) | 30 | 90 |
| $X_2$ = Surfactant (mg) | 20 | 60 |
| $X_3$ = Solvent (ml) | 1 | 3 |
| **Dependent variables** | | |
| Y1=Particle size (nm) | | |
| Y2 = Entrapment efficiency (%EE) | | |
| Y3=Polydispersity index (PDI) | | |