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Optimization of proniosomal itraconazole formulation using Box Behken design to enhance oral bioavailability

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ABSTRACT

The aim of our investigation was to study the combined impact of 3 independent variables on the development of itraconazole (ITR) proniosomes by using Box-Behnken design (BBD). BBD was implemented with 3 variable factors at 3 levels. The values of the factors and responses were exposed to multiple regressions to derive a second-order polynomial equation used to predict the values of optimized dependent variables (responses). The surfactant concentration, drug concentration and molar ratio (Span 60: cholesterol) were chosen as the independent variables. Fifteen proniosome formulae were prepared using the slurry method and estimated for drug entrapment efficiency (DEE), vesicle size (VZ) and % drug released after 8 h. The insignificant terms of the equation that showed the probability value ($P > 0.05$), were excluded from the full-model equation to attain a reduced-model equation to ease expecting the different responses upon changing variables. The effect of the different variables was also shown by the construction of line and contour plots. Two optimized formulae; P1 (0.28, 0.91 and −0.91) for the maximized response of DEE and % drug released without restriction of VZ and P2 (0.5, −1, −1) with a minimum VZ were prepared and evaluated for *ex-vivo* permeation study. *In-vivo* pharmacokinetic study of the optimized selected formula P2was tested against the market product (sporonox®) in rabbits. ITR Proniosomal formula showed 1.95 fold, 1.3 fold and 1.4 fold increase in C_{max}, $AUC_{0\text{-}48}$, and $AUC_{0\text{-}\infty}$, when compared to ITR market product. Our investigation suggested the potential use of proniosome as a drug carrier to enhance the bioavailability of ITR.

1. Introduction

Itraconazole is a triazole broad-spectrum antifungal agent. It has a great activity against different fungi such as sporotrichosis, histoplasmosis, *Aspergillus*, blastomycosis, and onychomycosis. Itraconazole is a weakly basic compound having pKa of 3.7. It can only be ionized at low pH such as in gastric juice with a very poor aqueous solubility. It was reported that the solubility of ITR in water, simulated gastric fluid, and simulated intestinal fluid was 2 ng/ml, 3.9 μg/ml, and 3 ng/ml, respectively [1].

Conventional ITR dosage form (ITR capsule) has a quite low bioavailability (~55%) after oral administration, especially when taken on an empty stomach as it is highly lipophilic [2]. This bioavailability reaches to the maximum when taken with a full meal. Various approaches increased the bioavailability of ITR but they have several disadvantages. For example, ITR complexes with hydroxypropyl beta-cyclodextrin (HP-β-CD) caused osmotic diarrhea in patients which could be attributed to the adjuvant HP-β-CD, not due to the drug toxicity [3]. The clearance of HP-β-CD reduced by 6-fold after given a single IV dose of ITR (200 mg) to patients with severe renal dysfunction when compared with persons having a normal renal function [4]. Therefore it is not endorsed in patients with renal dysfunction and elder people. Encapsulation of the ITR in vesicular structures like niosomes is one of such techniques that control the drug delivery rate, which results in prolonging the therapeutic activity duration or help in the delivery of the drugs to a target tissue and thus improving drug bioavailability [5]. Provesicular systems like proniosomes are the dry preparation. It is formed by coating a water-soluble carrier with a surfactant. This dry formulation can be rehydrated by gentle agitation in hot water. Dry Proniosomes overcomes the drawbacks of physical stability of noisome such as vesicle aggregation or fusion and leakage or hydrolysis of the drug. The ease of transportation, distribution, storage and dosing are other advantages of this delivery system [7,8]. Free flowing powders of proniosomes are prepared using a carrier like Mannitol or Maltodextrin, which coated with the lipid or surfactant solution in an organic solvent to form a thin surfactant film on the carrier.

Previous studies revealed the enhancement effect of noisome on the absorption of the drug from GIT and extend its existence in the systemic

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circulation [9,10]. Therefore, the ITR encapsulation in the lipophilic vesicular system may improve the oral absorption and bioavailability regardless food [11].It was reported that the bioavailability of drug encapsulated in niosomal system enhanced by passage the GIT barrier through transcytosis of M cells of Peyer's patches at the GIT lymphatic system [12].

The purpose of this study was to design and develop a novel optimized ITR proniosome formulation using Box-Behnken design 7 [13] to avoid the side effects of the conventional dosage form and then evaluate the impact of proniosomal formulation on bioavailability compared with ITR market tablet.

2. Materials and methods

Itraconazole (ITR) was received as a gift from the Eva-pharm Company, Egypt. Span60 and cholesterol were purchased from Sigma-Aldrich, USA. Mannitol (average particle size 250 μm) was purchased from El-Gomhoria Company, Egypt. All chemicals, used in the study were of analytical grades.

2.1. Fourier transmission infrared spectroscopy (FTIR)

FTIR was performed for estimating the possible physical interactions between ITR, span 60, cholesterol, and Mannitol. The compatibility study was done using an IR spectrophotometer (Prestige, Japan) by measuring the transmittance from 4000 to 400 cm^{-1}. Matching between peaks was carried out to recognize any probable interactions between ITR and the others additives (6).

2.2. Box-Behnken Experimental Design

Box-Behnken statistical design (BBD) with 3 factors (molar ratioofspan60: cholesterol; X_1, surfactant amount; X_2, and drug amount; X_3), and with 3levels (−1, 0, and 1) was built up to estimate the significance effect of these different variables on the responses namely, VZ, DEE and % drug release, and then predicts the optimized proniosomal formulae. BBD is appropriate to explore quadratic response surfaces and assemble second-order polynomial equations. The design includes sets of points in the middle of each side and three replicated center points of the multidimensional cube. The polynomial equation (Eq. (1)) was derived using a Design Expert software program (Version 7, Stat-Ease Inc., Minneapolis, MN). The dependent and independent variables were tabulated in Table 1.

$$Yi = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_2^2X_2^2 + b_3\,3X_3^2 \quad (1)$$

Where Y_i is the dependent variable; b_0 is the intercept; b_1 to b_3 are regression coefficients: and X_1, X_2, and X_3 are the independent variables.

Table 1
Variables and their levels in Box-Behnken Design.

Independent Variables	Levels		
	Low	Medium	High
X_1 = molar ratio of span 60: cholesterol	2:1	1:1	1:2
X_2 = surfactant amount	1 ×[a]	3 ×	5 ×
X_3 = drug amount	50 mg	100 mg	150 mg

Dependent variables Y1 = vesicle size, Y2 = % of drug entrapment, Y3 = % drug released after 8 h.

[a] 1 × corresponds to 1 mmol per gram of carrier (Mannitol).

2.3. Preparation of proniosomes

The slurry method was used to prepare proniosomes [9,14]. In brief, specific amounts of ITR, span60, and cholesterol were dissolved in 3 ml of a mixture of chloroform: ethanol (2:1). The mixture was heated to 45 °C with sonication until complete dissolution. Then, it was placed in 250 ml round-bottom flask containing Mannitol powder as a carrier. In case of the formulation with low surfactant amount, an additional volume of the solvent mixture was put to obtain slurry. The flask was connected to a rotary evaporator (RotavapR-300, Buchilabortechnic; Switzerland) which was operated at 50 rpm, a temperature of 40 ± 2 °C under a reduced pressure of 500 mmHg to evaporate the solvent mixture. The apparatus left to rotate up to 15 min to obtain a dried thin film onto the Mannitol particles. These dried proniosomal powders were kept in a tightly sealed container for further estimation. All formulae prepared according to the BBD were represented in Table 2.

2.4. Preparation of niosomes from proniosomes

The proniosomal powder of each formula was transformed into niosomal suspension by hydrating with 25 ml hot distilled water (80 °C). This suspension was agitated using a vortex mixer (Stuart vortex mixer, USA) for 2 min, then sonicated again twice for 30 s using a sonicator (Raypa, UCD-200). Niosomes was estimated for VZ and DEE.

2.5. Characterization of the prepared proniosomes

2.5.1. Vesicle size measurement (VZ)

Average vesicle size was measured using a Malvern particle size analyzer, (Malvern Mastersizer 2000 instruments Ltd., UK). 5 ml of each niosomal suspension was dispersed in 500 ml of double distilled water under gentle stirring (600 rpm) in a glass beaker. All measurements were means of triplicate (mean ± SE).

2.5.2. Determination of drug entrapment efficiency (DEE %)

The entrapment efficiency percent (DEE %) of ITR in the prepared proniosome was estimated directly by assaying the ITR content in the nanovesicles. Samples of 10 ml of the niosomal suspension were centrifuged at 7000 rpm for 1 h at 4 °C using a cooling centrifuge (Beckman, model TJ-6 provided with a refrigeration unit, UK). The supernatant was discarded and the residue was washed using 1% Tween 80 solution to get rid of any surface drug. After that, the dispersion was re-centrifuged and the residue was separated. The residue was dissolved in a mixture of ethanol and chloroform (50:50) (v:v) and estimated by using a validated HPLC method. Agilent column (250 × 4.6 mm, 5 μm) was used for chromatographic separation with a flow rate of 1.5 ml/min at a wavelength of 262 nm. Chromatography was performed at ambient temperature, with the mobile phase composed of acetonitrile: 0.25% ammonium acetate: methanol (3:1:1). The HPLC was validated for linearity, accuracy, precision, and LOQ, LOD and system suitability.

2.5.3. In-vitro release of ITR proniosomes

The dissolution study was performed using dissolution apparatus II (Hanson Research, SR8 plus, USA), at 50 rpm, 37 ± 0.5 °C and 500 ml 0.1 N HCl (pH 1.2) as the media of dissolution. A weighed amount of proniosome powder equivalent to 50 mg ITR and 2 ml dissolution medium was put in a cellulose dialysis tube (MW cut-off of 12000–14000) which was previously soaked in 0.1 N HCl. The two ends of the tube were joined firmly and tied to the paddle shaft of the apparatus [15]. Aliquots of 2 ml were withdrawn at different time intervals up to 24 h and filtered using 0.1 μm syringe filter. Replacements with an equal volume of fresh dissolution media at 37 °C were done. The %ITRreleasedwas estimated using the mentioned HPLC method. The estimation was done using a calibration curve of ITR in the