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# Zero order delivery of itraconazole via polymeric micelles incorporated in situ ocular gel for the management of fungal keratitis

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ABSTRACT

The aim of this article is to investigate the role of amphiphilic block copolymer-based polymeric micelles of itraconazole for the management of fungal keratitis to overcome the limitations of the conventional dosage form. The polymeric micelles were made using pluronics above critical micelle concentration. Itraconazole-loaded polymeric micelles prepared by rotary evaporation method were characterized and the optimized micellar formulation (M5) was selected on the basis of least micelle size (79.99 nm), maximum entrapment efficiency (91.32% ± 1.73%) and in vitro permeation (90.28% ± 0.31%) in 8 h, that best fitted zero-order kinetics. M5 was developed as pH sensitive in situ gel and characterized for various parameters. The optimized in situ gel (F5) proved to be superior in its ex vivo transcorneal permeation when compared with Itral® eye drop and pure drug suspension, exhibiting 41.45% ± 0.87% permeation with zero-order kinetics ($r^2$ = 0.994) across goat cornea. Transmission electron microscopy revealed spherical polymeric micelles entrapped in the gel matrix. A spectrum of tests revealed hydration capability, non-irritancy, and histologically safe gel formulation that had appropriate handling characteristics. Conclusively, a controlled release pH-sensitive ocular formulation capable of carrying drug to the anterior segment of the eye via topical delivery was successfully developed for the treatment of fungal keratitis.


## 1. Introduction

Keratitis is the inflammation of cornea characterized by corneal edema, cellular infiltration, and cilliary congestion, and can be both infectious and non-infectious. The occurrence of keratitis varies depending on the cause of infection, which can be bacterial, fungal, or viral. Out of these, the fungal infection is the most common. The fungi more commonly responsible for mycotic corneal ulcers are *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Candida*, and *Fusarium* [1]. The fungus can invade into the stromal tissue through a defect in the epithelial sheet, after which it invades into corneal stroma and multiplies, with subsequent tissue necrosis and triggers a host inflammatory response. Unfortunately, these organisms may penetrate through an intact Descemet's membrane and enter the anterior chamber. Therefore, eradication of these pathogens becomes more difficult to treat [2]. The antifungal agents available for treatment of fungal keratitis are generally associated with poor corneal penetration, stromal permeability, and poor clinical outcomes [3].

Itraconazole used for the management of fungal keratitis is a synthetic triazole agent and has pronounced antifungal activity against *Aspergillus*, *Curvularia*, and *Candida* species. Commercially available as Itral® eye drops (Jawa Pharmaceuticals, Gurgaon, India) is applied hourly and is precipitated in the corneal tissue after topical administration [4]. The formulation is also associated with poor corneal penetration and visual disturbances, lacrimation, tear dilution, nasolacrimal drainage, and tear turnover. Hence, it was aimed to investigate an amphiphilic block copolymer-based polymeric micellar ocular delivery system with superior corneal penetration to combat fungal invasion in the anterior chamber of eye devoid of irritation issues.

Amphiphilic block copolymers have the affinity to self-assemble into micelles above the critical micelle concentration to form nano-sized (10–100 nm) carrier with a core–shell structure. The hydrophobic core surrounded by hydrophilic shell offers entrapment of hydrophobic drug that serves as drug reservoir while hydrophilic shell forms steric barrier to micelles aggregation and ensures micelle solubility in aqueous environment [5]. The polymeric micellar delivery system of itraconazole is expected to improve corneal permeability and control its release at the target site.

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## 2. Materials and methods

### 2.1. Materials

Itraconazole was obtained as a gift sample from Ranbaxy Pvt. Ltd., Gurgaon, India. Poloxamer 407 (Pluronic F127) and Poloxamer 188 (Pluronic F68) were procured from BASF Corporation, NJ, USA. Methanol, tetrahydrofuran (THF), sodium chloride, sodium hydroxide pellets were obtained from Qualigens Fine Chem. Pvt. Ltd., Mumbai, India. Carbopol 943 P was purchased from Central drug house Ltd., New Delhi, India. Sudan IV dye AR and dialysis membrane 150 were purchased from Hi Media Laboratories, Mumbai, India.

### 2.2. Methods

#### 2.2.1. Critical micelle concentration

Critical micelle concentration (CMC) of Pluronic F127 (PF127) and pluronic F68 (PF68) was determined by dye micellization method using Sudan IV dye. Dilutions in the concentration range of 1–20 mg/mL were prepared from stock solution(s) of PF127 and PF68. One milliliter of Sudan IV in chloroform (1%, w/v) was added to 5 mL of each dilution. The mixture was shaken and the aqueous layer collected was centrifuged for 1 h at 1000 rpm. The samples were analyzed spectrophotometrically (Pharm Spec 1700, Shimadzu, Kyoto, Japan) at 350 nm. The CMC was determined at the inflection point on the plots representing absorbance as a function of the copolymer concentration [6].

#### 2.2.2. Itraconazole-loaded polymeric micelles

Itraconazole-loaded polymeric micelles were prepared by rotary evaporation method [7]. The drug and copolymer were dissolved in nonselective solvent methanol/THF separately, according to formulation design given in Table 1. The solution was stirred at room temperature for 1 h and the solvent was allowed to evaporate at 45 °C under vacuum in rotary evaporator (Hicon®, New Delhi, India). The thin film formed was hydrated with 50 mL distilled water previously heated at 60 °C and mixture was stirred vigorously at 37 °C with continuous rotation of 100 rpm until polymeric micelles containing itraconazole were formed. Dialysis against water was performed in a dialysis bag (Mol. Wt. cut off 12–16 kDa) to remove un-entrapped drug. The purified dispersion was filtered through 0.45 μm Cellulose nitrate filter paper (Sertorrius A.G. 37070, Goettingen, Germany) to remove microparticulate impurities and the filtrate contained pure colloidal suspension of itraconazole-loaded polymeric micelles.

#### 2.2.3. Entrapment efficiency

One milliliter of the formulation was dissolved in 5 mL methanol and vortexed to ensure complete micelle destruction and hence drug release. The volume was made up to 10 mL with simulated tear fluid (STF) (pH 7.4) (consisted of 6.78 g of NaCl, 1.38 g of KCl, 2.18 g of $NaHCO_3$ and 0.0843 g of $CaCl_2$ dissolved in deionized water, volume made up to 1l and the pH was adjusted to 7.4) and percentage of entrapment efficiency was calculated by the following Eq. (1),

$$\%\,EE = \frac{\text{Amount of drug present in 5 mL micelles formulation} \times 100}{\text{Amount of drug initially added}} \quad (1)$$

#### 2.2.4. Micelles size and polydispersity index

The micelle size and polydispersity index (PDI) were determined by light scattering based on laser diffraction using the Malvern Zetasizer (Malvern Co., UK). The scattering angle was fixed at 173° and temperature was maintained at 25 °C. Each sample was diluted with deionized water and filtered through millipore 0.45 μm pore size membrane (Milex®, Millipore Co., USA) before determination at 25 °C using clear disposable sizing cuvette.

#### 2.2.5. In vitro drug permeation

A fabricated Franz diffusion cell consisting of donor and receptor compartments separated by presoaked dialysis membrane (pore size 0.22 μm) was used for the study. The formulation equivalent to 10 mg/mL of itraconazole-loaded polymeric micelles was placed in the donor compartment and the receptor compartment was filled with STF, pH 7.4 stirred at 100 rpm using magnetic stirrer. At predetermined time intervals, 1 mL sample was withdrawn and replaced with same volume of fresh medium. The drug concentration of each sample was measured and plots of percentage of cumulative drug permeated were made as function of time.

#### 2.2.6. Selection of optimized micellar formulation

The optimized formulation was selected on the basis of the highest percentage of entrapment efficiency, percentage of cumulative drug permeated with optimum particle size, and lowest PDI value, and characterized by transmission electron microscopy (TEM).

#### 2.2.7. Transmission electron microscopy

The negatively stained optimized sample (M5) was air dried at room temperature and TEM image was obtained using FEI Technai $G^2$ TEM grid (FEI, Houston, USA) operating at an acceleration voltage of 200 kV.

#### 2.2.8. Itraconazole-loaded polymeric micellar in situ gel

Gel base solutions were prepared by dispersing carbopol 934P in deionized water with continuous stirring until it gets completely dissolved and finally the volume was adjusted to obtain gel base strength(s) of 0.1–0.5% (w/v). Formulation (M5) equivalent to 10 mg/mL of itraconazole was subjected to cooling centrifugation (REMI Instrument Ltd, Vasai, India) at 14,000 rpm for 15 min and the pellets obtained were incorporated into 10 mL in situ gel base. The formulations were coded and evaluated as described below.

#### 2.2.9. pH, clarity, and drug content

The clarity of the developed gel formulations was determined before and after gelation by visual examination of the formulations under light, alternatively against white and black background. The pH of the developed gel was determined using digital pH meter model 111 E (Hicon®, New Delhi, India). For drug content, weighed amount (100 mg) of in situ gel was diluted with 5 mL methanol. The resultant dispersion was vortexed for 10 min, volume made up to 10 mL with methanol, filtered through nylon filter and analyzed spectrophotometrically at 262 nm.

#### 2.2.10. In vitro gelation

The formulations were evaluated for in vitro gelation to identify the composition that was best suited for use as in situ gel. The in vitro gelling efficiency was determined by mixing the in situ gel with STF, pH 7.4 in the ratio 25:7 (25 μL of in situ gel and normal tear volume 7 μL) to mimic the in vivo ocular conditions equilibrated at 37 °C ± 0.5 °C. The gelation was assessed visually, and the time required for gelation was recorded.

#### 2.2.11. Optimized in situ gel

Optimized in situ gel was selected on the basis of best clarity, maximum gelling capacity, and highest drug content, and characterized for a battery of tests appropriate for formulations intended for ocular administration.