

# Smart *in-situ* thermo-responsive and ion activated ophthalmic sol-gel system of fluconazole

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**ABSTRACT:** The objective of the current investigation is the development and characterization of *in-situ* ophthalmic formulation of fluconazole for sustained release at the eye site for a prolong period of time. Sol-gel fluconazole *in-situ* formulation was prepared by thermo-responsive pluronic F127, Na<sup>+</sup> activated sodium alginate alone and in combination, characterization parameters include FTIR spectroscopy, Visual assessment and clarity test, Gelling ability test, pH testing, drug assay, *In-vitro* drug release and optimized formulation was evaluated for *In-vitro* antifungal studies by comparing with marketed formulation of fluconazole. Optimized formulation F8 composed of fluconazole (0.3%w/v), Pluronic F127(1%w/v), sodium alginate (0.5%w/v), sodium chloride (0.9w/v), benzalkonium Chloride (0.01%w/v) and acetate buffer pH 4 up to 100%w/v), showed drug-polymer compatibility as per FTIR, high gelling consistency on contact with simulated physiological conditions, 6.9 pH, drug assay was estimated (99.89±0.78 %) with 100% *in-vitro* drug released for about 6 hours. The *in-vitro* antifungal study was found to be 18.87±0.65 mm; 20.76±0.23 mm in comparison to marketed fluconazole conventional gel formulation (15.98±0.98 mm; 18.98±0.76 mm) for *Candida albicans* and *Aspergillus niger* pathogenic fungal strains respectively. Prepared *in-situ* ophthalmic sol-gel formulation of fluconazole could be considered as efficient delivery system to sustained the drug at the target site and effectively eradicating deeply rooted pathogenic fungal stains.

**KEYWORDS:** Thermos-responsive; Na<sup>+</sup> activated; sol-gel; fluconazole; *Candida albicans*.

## 1. INTRODUCTION

Special sense organ eye should be dealt with great attentiveness, while delivering the drug. Anatomical position and physiological function of eye hindered most of the conventional drug therapies leading to failure in attaining the desired drug concentration [1]. Continuous movement of eyeball (3-5/sec or over a million times/day) besides lacrimation flow rate of lubrication about (170-260 ml/min) and posture of body could influence the absorption of drugs [2]. Cornea the outermost protective layer composed of five layers serves as barrier against, dirt, microbes and protect the eye from hazardous effects of radiations to some extends [3]. Lipoidal cells (90%) of cornea possess significant resistance for topically applied drugs [4]. Pharmacokinetics of ophthalmic dosage explains the conventional dosage of eye drop 5 equivalent of 0.25ml distributes in 7 µL of tear fluid leading to 47 µL volume of distribution (Vd) with elimination rate constant of 0.02 min<sup>-1</sup> [5]. Lacrimal drainage also reduces the active concentration of the drug at the required site. The aforementioned factors emphasis the failure of conventional eye drops to achieve therapeutic efficacy. Superficial eye fungal infection more predominant with frequent reoccurrence [6]. Infection at the eye site considered to be one of the main causes for keratitis, endophthalmitis, dacryocystitis and orbital cellulitis results in ocular morbidity followed by impaired or loss of vision [7]. Antifungal drugs widely used for treatment, their administration in eye drops couldn't be able to eradicate the fungal infection completely [8]. Fluconazole considered being the drug of first-line therapy for many ocular fungal infections caused by *Candida* and *Tinea* pathogenic species [9]. Formulation scientist has applied novel drug delivery and nano-technologies in order to conquer the barriers to achieve desired drug concentration and target drug to the site of action [10]. First controlled pilocarpine delivery Ocusert® was fabricated for management of glaucoma conditions, permeation enhancers β-cyclodextrin employed to increase the solubility and increase permeation, drug encapsulated noisome, liposome, polymeric nanoparticles and nanocrystals were also applied to temporal and targeted delivery of drug at the eye site [11,12]. *In-situ* ophthalmic delivery systems were considered to be patient compatible, retained at the eye site for prolong period of time thereby increasing the contact time, absorbtion and

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bioavailability of the drug. The drug presented in solution physical state offered easy accessible and dose administration that on contact with the eye site due to the physiological temperature and alkaline environment the instilled solution turns to gel, henceforth called sol-gel systems.

## 2. RESULTS AND DISCUSSION

### 2.1. FTIR spectroscopy

FTIR spectrum of FLZ showed in fig-1 represents O-H stretching vibrations at  $3350\text{ cm}^{-1}$ , aromatic C-H stretching vibrations at  $3000\text{ cm}^{-1}$ , aromatic C=N stretching vibrations at  $1650\text{ cm}^{-1}$  and aromatic C-F stretching vibrations at  $1000\text{--}1400\text{ cm}^{-1}$ . Most of these fingerprints region peaks of fluconazole get shifted and shorten in the formulation composed drug and polymers, representing no chemical interaction of drug with the polymers and considered to be the compatible (Figure 1).

### 2.2. Visual assessment and clarity test

The evaluation parameters revealed; sol-gel systems prepared by pluronic F-127 (F1-F3) were transparent and cleared in contrast to sodium alginate sol-gel formulation light yellow in color with pale yellow and cloudiness in F6. Visual appearance and clarity test showed a light yellow color and cloudiness for formulation F6 and F9. Sol-gel F8 formulation showed transparency. Results are given in Table 1.

### 2.3. Gelling ability test

Gelling ability was found to increase with increase in the polymer concentration, formulation F3, F5, F6, F8 and F9 indicated immediate and extended gelation (Table 1).

### 2.4. pH testing

All the formulation had the biocompatible pH suitable for installation in the eye, without causing any irritation. Results are given in Table 1.

### 2.5. Drug assay

Drug content estimation was found to be within the prescribed limits of official compendia. Results are given in Table 1.

### 2.6. *In-vitro* drug release

*In-vitro* drug release was decreased with an increase in the polymer concentration, that could be attributed due to an increase in viscosity, which may hinder the drug molecule to permeate in the diffusion medium. Percentage FLZ released found to be 99.99%, 94.11%, 89.99% for F1-F3 respectively, Figure 2. Sol-gel the formulation prepared by sodium alginate (F4-F6) showed decrease released in comparison to the pluronic-127 formulation (F1-F3), Figure 3. Formulations F7-F9 showed relatively sustained drug released due to synergistic thixotropic property of pluronic F-127 and sodium alginate, Figure 4. From *in-vitro* drug release study F8 formulation was considered to be an optimized showed 100% drug released in 6 hours. Drug release study could be correlated with the results reported by Üstündağ Okur *et al* [13].

### 2.7. *In-vitro* antifungal studies

Formulation, F8 was considered for further testing its efficacy against the pathogenic fungal strains by *in-vitro* antifungal study, Figure 5. Antifungal study revealed zone of inhibition of FLZ as positive control ( $12.87\pm 0.89\text{ mm}$ ,  $18.65\pm 0.87\text{ mm}$ ) for *C. albicans* and *A. niger* respectively. Comparison of marketed FLZ gel ( $15.98\pm 0.98\text{ mm}$ ;  $18.98\pm 0.76\text{ mm}$ ) and optimized F8 showed ( $18.87\pm 0.65\text{ mm}$ ;  $20.76\pm 0.23\text{ mm}$ ) for *C. albicans* and *A. niger* respectively.

## 3. CONCLUSION

Ophthalmic fungal infection, one of the persistent causes of ocular morbidity. The efficacy of treatment depends on the residence and localization of antifungal drugs in the corneal region. Optimized formulation F8 composed of both pluronic F-127 and sodium alginate converts the solution to gel by physiological temperature ( $37^\circ\text{C}$ ) and  $\text{Na}^+$  ions of lacrimal fluid. Formulation F8 sol-gel transition leads to an increase in viscosity, longer residence and sustained drug release with effectiveness against pathogenic strains (*C. albicans* and *A. niger*) make it suitable for the treatment of topical ophthalmic fungal infection.

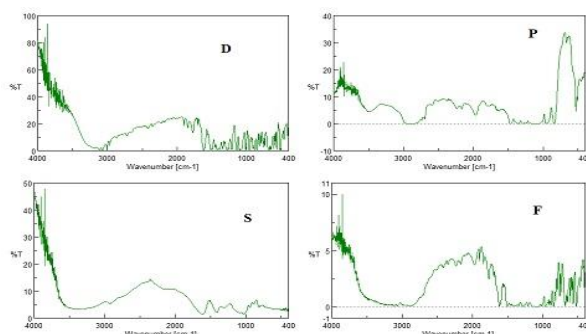


Figure 1. FTIR spectra of (D) FLZ, (P) pluronic F127, (S) sodium alginate and (F) formulation.

Table 1. Evaluation parameters of fluconazole *in-situ* ophthalmic sol-gel.

Formulation code	Evaluation Parameters				
	Visual appearance	Clarity	Gelling ability	pH	(%) Drug content
F1	Transparent	Clear	+	6.87	99.76±0.89
F2	Transparent	Clear	++	7	98.99±0.98
F3	Transparent	Clear	+++	6.7	99.08±0.78
F4	Light yellow	Clear	++	6.9	99.09±0.67
F5	Light yellow	Clear	+++	7.2	98.98±0.87
F6	Pale yellow	Cloudy	+++	7.4	98.78±0.99
F7	Light yellow	Cloudy	++	6.8	98.99±0.76
F8	Transparent	Clear	+++	6.9	99.89±0.78
F9	Light yellow	Cloudy	+++	7.1	98.89±0.98

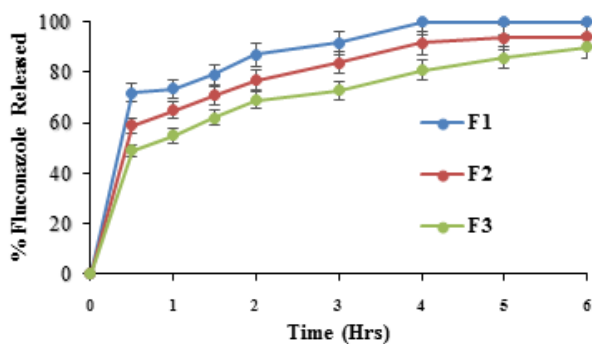


Figure 2. *In-vitro* drug release of Pluronic F-127 FLZ *in-situ* sol-gel.

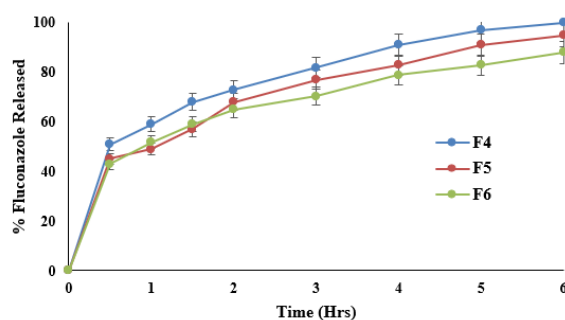


Figure 3. *In-vitro* drug release of sodium alginate FLZ *in-situ* sol-gel.

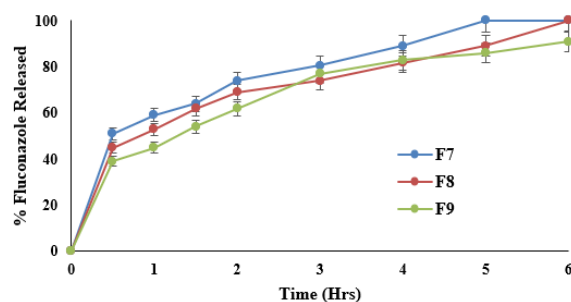


Figure 4. In-vitro drug release of Pluronic F-127 and sodium alginate FLZ in-situ sol-gel.

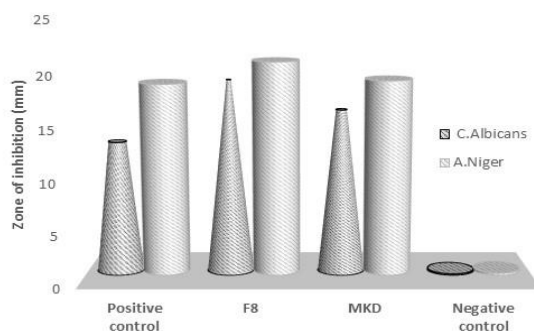


Figure 5. In-vitro Antifungal study of FLZ in-situ sol-gel.

#### 4. MATERIALS AND METHODS

Materials: Fluconazole was obtained as drug gift sample from JPI, Riyadh. Sodium alginate and Pluronic F-127 purchased from Sigma Aldrich, United States. Sodium Chloride and Benzalkonium chlorides were procured from Loba Chemie Pvt Ltd, India. All the other chemicals used were of analytical grades and used as its supplied without any purification and testing. Ultrapure water used was processed by Milli-Q® Integral Water Purification System.

##### 4.1. Preparation of fluconazole in-situ ophthalmic sol-gel

Drug - polymeric solutions were prepared by dissolving fluconazole (FLZ) 0.3%W/W and required concentration of sodium alginate (hot-aqueous phase), pluronic F127 (cold aqueous phase) in alone and combinations as per Table 2, using ultrapure water. Sodium chloride and Benzalkonium chloride solutions added in the above drug-polymeric solution by continuous mixing on the magnetic stirrer at 400 rpm with heating (50°C on ceramic plate (Thermo Fisher Scientific, Massachusetts USA). Final volume was made up to 100 mL and pH was adjusted with acetate buffer to pH 4 [14]. Entire process of preparation was done under aseptic conditions of BSC class II cabinets (LabGard ES NU-437S Class II Biosafety Cabinet, USA).

Table 2. Preparation of fluconazole in-situ ophthalmic sol-gel.

Composition (%W/V)	Formulation Code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Fluconazole	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Pluronic F127	0.5	1	1.5	-	-	-	0.5	1	0.5
Sodium Alginate	-	-	-	0.5	1	1.5	0.5	0.5	1
NaCl	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Benzalkonium Chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Acetate Buffer pH 4	100	100	100	100	100	100	100	100	100

## 4.2. Characterization of *in-situ* ophthalmic sol-gel

### 4.2.1. FTIR spectroscopy

FTIR analysis performed in the region of 4000 to 400  $\text{cm}^{-1}$  by the Fourier transformer spectroscopy technique (Perkin-Elmer spectrophotometer, Germany; model 2000 FTIR). Drug (FLZ) and polymer compatibility study was done by taking FLZ, and polymers; pluronic F127, sodium alginate alone and in combination with potassium bromide spectra were interpreted for possible physicochemical interactions [15].

### 4.2.2. Visual assessment and clarity test

In order to examine the formulation and confirm the negative grittiness and particulate matter. Prepared ophthalmic solutions were examined against black and white background [16].

### 4.2.3. Gelling ability test

The prepared formulation was dropped into vial (2mL) filled with simulated tear fluid maintained in physiological conditions. (Simulated tear fluid - STF composed of NaCl 0.67 g,  $\text{NaHCO}_3$  0.20 g,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  0.008 g, in deionized water to make 100 g). The gelling ability was visually observed, sol-gel transitions then noted [17].

### 4.2.4. pH testing

All the formulations were assessed for hydrogen potential using pH meter (3510 pH Meter, Jenway, UK). Formulation first dissolved in ultrapure water and electrode is dipped into sample and pH was noted.

### 4.2.5. Drug assay

All the formulation was evaluated for drug content estimation by diluting 2mL of sample up to 100mL of simulated tear fluid pH 7.4. The concentration of drug followed by % drug content was estimated by UV-spectrophotometric analysis (Jasco v-630 spectrophotometer, German) at  $\lambda_{\text{max}}$  261 nm [18].

### 4.2.6. In-vitro drug release

Drug diffusion study was performed in fabricated diffusion cell using cellophane membrane (Mol. Wt. 14 kDa). STF was filled up to 100mL into cell and maintained at  $37 \pm 1^\circ\text{C}$  with continuous stirring on thermostatically controlled magnetic stirrer (Thermo Fisher Scientific, Massachusetts USA). At predetermined time intervals, 1 mL samples were withdrawn and replenished with STF in order to maintain the sink condition. The samples were analyzed UV- spectrophotometric analysis (Jasco v-630 spectrophotometer) at  $\lambda_{\text{max}}$  261 nm and percentage drug released plotted against time [13, 19].

### 4.2.7. In-vitro antifungal studies

Anti-mycological study was performed by ditch plate technique using sabouraud agar culture medium. Optimized formulation F8, pure drug FLZ (+ control), marketed formulation (mkd) and blank as negative control. Fungal strains were streaked over the culture medium and incubated for 72 hours at  $25^\circ\text{C}$  in incubator [20-23]. Followed by measuring the one of inhibition the results were plotted for both fungal strains viz; blank *Aspergillus niger* and *Candida albicans*.

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