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Fluconazole Ocular Inserts: Formulation and *In -Vitro* Evaluation

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ABSTRACT:
The eye presents unique opportunities and challenges when it comes to the delivery of Pharmaceuticals. While absorption by this route is bungling, there are few side effects with conventional ocular dosage forms like eye drops and eye suspensions. Several polymeric systems have been used to fabricate ocular inserts for better ocular bioavailability and retention of drug for which gelling systems have shown advantages of convenient administration and increased contact time. Fluconazole ocular films were prepared using film forming polymers namely; Hydroxy propyl methyl cellulose, poly vinyl pyrrolidine and poly vinyl alcohol. PEG-400 was incorporated as plasticizer. Solvent casting technique was followed to prepare fluconazole ocular inserts. Seven formulations were formulated .The prepared ocular inserts were characterized by means of film thickness, weight variation, folding endurance and surface pH, and *in- vitro* drug release to determine the amount of drug release from selected film formulae using excised goat cornea. Ocular inserts prepared were smooth and passed all the evaluation tests performed. Formulation F5 shows a maximum cumulative percentage drug release of 69.02 % at the end of 2 hours through excised goat cornea. The drug in the films was found to be active against selected fungal species as was proved by microbial efficacy studies.

Keywords: *Fluconazole Ocular inserts, Solvent casting technique, Zero order release, In Vitro Evaluation*

Introduction

The eye as a portal for drug delivery is generally used for local therapy against systemic therapy in order to avoid the risk of eye damage from high blood concentrations of the drug, which is not intended. The unique anatomy, physiology and biochemistry of the eye render this organ impervious to foreign substances, thus presenting a constant challenge to the formulator to circumvent the protective barriers of the eye without causing permanent tissue damage [1].

Most ocular treatments like eye drops and suspensions call for the topical administration of ophthalmically active drugs to the tissues around the ocular cavity. These dosage forms are easy to instill but suffer from the inherent drawback that the majority of the medication they contain is immediately diluted in the tear film as soon as the eye drop solution is instilled into the cul-de-sac and is rapidly drained away from the precorneal cavity by constant tear flow and lacrimo-nasal drainage. Therefore, only a very small fraction of the instilled dose is absorbed by the target tissue for this reason, concentrated solutions and frequent dosing are required for the instillation to achieve an adequate level of therapeutic effect. One of the new classes of drug delivery systems, ocular inserts, which are gaining worldwide praise, release drugs at a pre-programmed rate for a longer period by increasing the precorneal residence time [2- 4].

The unique structure of the human eye as well as exposure of the eye directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by fungi. Host defenses directed against these microorganisms, once anatomical barriers are breached, are often insufficient to prevent loss of vision. Therefore, the timely identification and treatment of the involved microorganisms are paramount. For example, filamentous fungal infections of the eye are usually due to penetrating trauma by objects contaminated by vegetable matter of the cornea or globe or, by extension, of infection from adjacent paranasal sinuses. Fungal endophthalmitis and chorioretinitis, on the other hand, are usually the result of antecedent fungemia seeding the ocular tissue. Candida spp are the most common cause of endogenous endophthalmitis, although initial infection with the dimorphic fungi may lead to infection and scarring of the chorioretina. Contact lens wear is associated with keratitis caused by yeasts, filamentous fungi, and Acanthamoebae spp[5].

Standard initial treatment consists of frequent instillation of eye drops with a broad-spectrum antifungal agent. Fluconazole is a triazole antifungal drug used in the treatment and prevention of superficial and systemic fungal infections. In a bulk powder form, it appears as a white crystalline powder, and it is very slightly soluble in water and soluble in alcohol. Fluconazole inhibits the fungal cytochrome P450 enzyme 14α-demethylase. This inhibition prevents the conversion of lanosterol to ergosterol, an essential component of the fungal cytoplasmic membrane, and subsequent accumulation of 14α-methyl sterols[6]. Fluconazole is a prescription drug indicated for the treatment and prophylaxis of fungal infections where other antifungals have failed or are not tolerated (e.g. due to adverse effects), including Candidiasis caused by susceptible strains of *Candida,* Tinea corporis, Tinea cruris or Tinea pedis, Onychomycosis and Cryptococcal meningitis. Fluconazole can be used first-line for the Coccidioidomycosis, Cryptococcosis, Histoplasmosis and Prophylaxis of candidiasis in immunocompromised people [7]. Ocular therapy in the fungal infections would be significantly improved if the precorneal residence time of drugs could be increased. Successful results have been obtained with inserts and collagen shields [8]. Several polymeric systems are investigated to fabricate ocular inserts for better ocular bioavailability and retention of drugs [9].
In the present study, it was aimed to prepare and evaluate ocular films containing fluconazole along with hydrophilic and hydrophobic polymers either alone or in combination at different concentrations with better solubility and longer duration of action delivering the drug in zero order kinetics

Materials and Methods

Materials

Poly Vinyl Alcohol was acquired from S.D.Fine – Chemicals Ltd., (Mumbai). PVP K-30 was a gift sample from Central Drug House (New Delhi), HPMC K 100 was acquired from Lab Chemicals (Chennai), Glycerin purified was acquired from Loba Chemicals Ltd (Mumbai), Fluconazole (FL) was obtained from Hetero Drugs Limited (Hyderabad)., All other reagents and solvents were of analytical grade and used as received.

Preparation of Ocular inserts

The ingredients for preparation of fluconazole ocular inserts were weighed as shown in the table -1 for F-1, F-2, (PVA alone); F-3, F-4, F-5, the combination of polyvinyl alcohol and polyvinyl pyrrolidine (k-30) was used .the volume of PVA used was kept constant but the volume of PVP (k-30) was increased. PVA, PVP (K-30) and fluconazole were mixed well and the volume was made up to 100 ml with water .The above was sonicated in an ultrasonicator at a speed of 80 MHz for 20 mins. After dispersion, 0.2 ml of propylene glycol 30% w/v was added as a plasticizer.

The ingredients were weighed as shown in the table -1 for F-6, F-7 HPMC (K-100), glycerin and propylene glycol were used. HPMC (K-100), glycerin, propylene glycol and fluconazole were mixed well and made up to 100 ml with water and sonicated in an ultrasonicator at 80 MHz for 15 min. This is allowed to stand over night to remove entrapped air bubbles. The solutions were poured into glass molds by placing them on flat surface and the solvent was evaporated by in hot air oven at 35 ± 2^0C for 3 hrs. Dried ocular films were carefully removed and cut into square films with the help of a sharp edged die [10].

Evaluation of the fluconazole ocular inserts / films:

1. **Identification of fluconazole in films:** Fluconazole was identified by IR spectral analysis (Perkin Elmer 1720 FTIR). Fluconazole discs were prepared by pressing Fluconazole with potassium bromide and the spectrum was recorded between 4000 cm^{-1} to 500 cm^{-1}. The absorption maxima in the spectrum obtained with the substance being examined was compared with that of reference spectrum.

2. **Weight Uniformity:** For determination of film weight uniformity, Six films of one square centimeter for each formulation were randomly selected and weighed individually on electronic balance (AND HR 2000). Mean weight of inserts *of* each formulation was recorded. The mean and standard deviation (S.D) were then calculated [11]. Data given in Table-2
3. **Thickness of Insert:** Thickness was measured at six different points on the film using micrometer and average was taken as the thickness of the film. Six such films of each formulation were measured to determine the thickness of the films[11]. Mean and SD were calculated. Table -2.
4. **Surface pH Determination:** Inserts were left to swell for 5 hours on agar plate prepared by dissolving 2% (m/v) agar in warm simulated tear fluid (STF; sodium chloride: 0.670g, sodium bicarbonate: 0.200 g, calcium chloride.2H2O: 0.008 g, and purified water q.s. 100 g[12] of pH 7.2 under stirring and then pouring the solution into Petri dish till gelling at room temperature. The surface pH was measured by means of a pH paper placed on the surface of swollen patch. Data produced in table-2
5. **Folding endurance value**: The folding endurance is expressed as the number of folds (number of times the insert is folded at the same place, either to break the specimen or to develop visible cracks. This test is important to check the ability of the sample to withstand folding. This also gives an indication of brittleness. The specimen was folded in the center, between the fingers and the thumb and then opened. This was termed as one folding. The process was repeated till the insert showed breakage or cracks in center of insert. The total folding operations were named as folding endurance value [13]. Values shown in table -2
6. **Uniformity of fluconazole content:** Uniformity of the drug contents was determined by assaying the individual inserts. Each insert of one square centimeter was grounded in a glass pestle mortar and to it was added 5 ml of methanol was added to make a suspension. The suspension so obtained was filtered and the filtrate was assayed spectrophotometrically at 260 nm. (UV-VIS Systronics Spectrophotometer-106)[13]. Values shown in table -2
7. ***In-vitro* transcorneal permeation studies**: Whole eye ball of goat was transported from local butcher shop to the laboratory in cold (4 °C) normal saline within 1 hour of slaughtering the animal .The cornea was carefully excised along with 2 to 4 mm of surrounding scleral tissue and was washed with cold normal saline till the washing was free from proteins. Isolated cornea was mounted by sandwiching surrounding scleral tissue between clamped donor and receptor compartments of an all glass modified Franz diffusion cell in such way that its epithelial surface faced the donor compartment. The receptor compartment was filled with 15 ml of freshly prepared STF. One square cm of ocular film was placed on the cornea and opening of the donor compartment was sealed with a glass cover slip, while the receptor fluid was maintained 35^0C with constant stirring, using Teflon coated magnetic stir bead. Three ml sample was withdrawn from receptor compartment at various time intervals up to 120 min and was analyzed spectrophotometrically at 260nm. Each sample withdrawn was replaced with equal volume of STF [14]. Values shown in table -3
8. ***In-vitro* antimicrobial efficacy**: A filter paper disc method [15] was employed for the *in vitro* study of antifungal effects against *Candida albicans*. The filter paper disc method was performed using Sabouraud dextrose broth and Mueller Hinton broth. These agar media were inoculated with 0.5 mL of the 24 h liquid cultures containing 10^7 microorganisms/mL. Ocular films instead of Filter paper discs were placed on the indicated agar mediums. The incubation time was 48h at 30°C for *Candida* species. The diameter of zone of inhibition was measured by using an antibiotic zone finder.

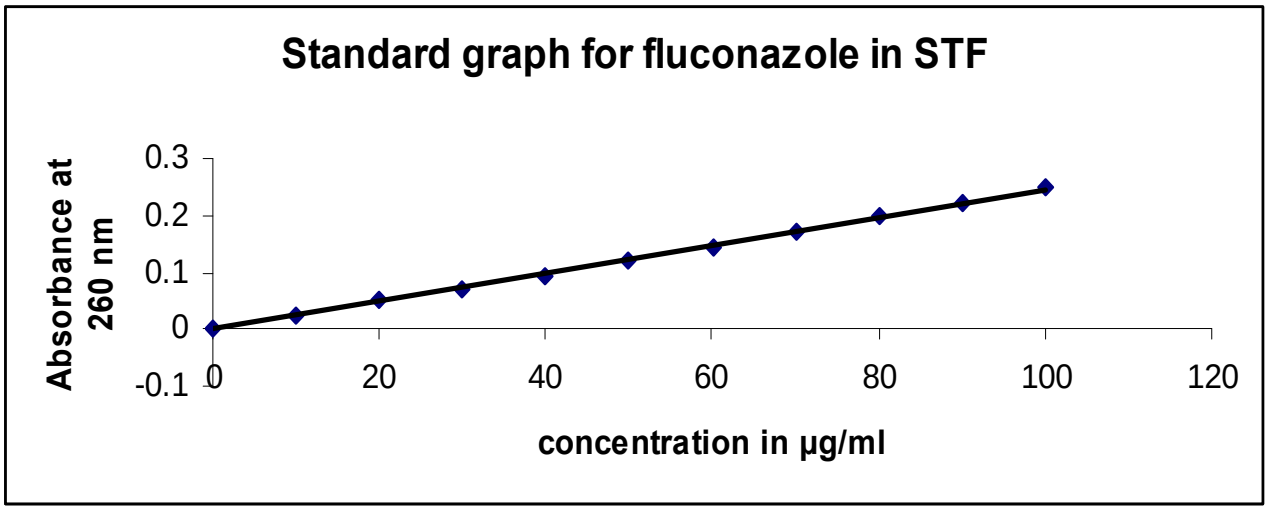


Fig 1

Table: - 1 Composition of fluconazole ocular films

Ingredients % (w/v)	Polyvinyl Alcohol Gms	Polyvinyl Pyrrolidine K-30 Gms	Hydroxy Propyl methyl Cellulose Gms	Propylene Glycol Ml	Glycerin Gms	Water Q.s to Ml	Fluconazole (Gms)
F-I	4	-	-	0.2	-	100	0.3
F-2	5	-	-	0.2	-	100	0.3
F-3	4	1	-	0.2	-	100	0.3
F-4	4	1.5	-	0.2	-	100	0.3
F-5	4	2.5	-	0.2	-	100	0.3
F-6	-	-	2	4.98	2.5	100	0.3
F-7	-	-	3	4.98	2.5	100	0.3

Results and Discussion

Fluconazole was identified by IR spectral analysis. The IR spectrum of the sample was found to comply with the spectrum obtained from reference sample. The calibration curve of fluconazole in methanol was derived from the concentration and the corresponding absorbance values. Linear regression analysis (LRA), gave the equation for the line of best fit as, y = 0.0026x + 0.02 Absorbance data was given in fig. 1. Calibration curve of fluconazole was done in STF and the LRA yielded the equation = 0.002x + 0.02 as line of best fit. Absorbance data was given in fig. 1

Totally seven formulations were prepared and named as F-1 to F-7. F-1 and F-2 contains only PVA, F-3 to F-5 contain combination of PVA and PVP (K-30), F-6 and F-7contain HPMC (K-100). Propylene glycol, glycerin were used for a plasticizing effect. Water used as medium for preparation. Fluconazole dosage fixed at 0.3%w/v. The preparation of fluconazole ocular films was executed as per procedure and formula as given in table-1.

Absorbance data of fluconazole in pH 7.4 phosphate buffer

Concentration	Absorbance
0	0
10	0.026
20	0.05
30	0.072
40	0.095
50	0.119
60	0.143
70	0.17
80	0.197
90	0.222
100	0.248

Table: - 2 Physicochemical characterization of Fluconazole Ocular Films

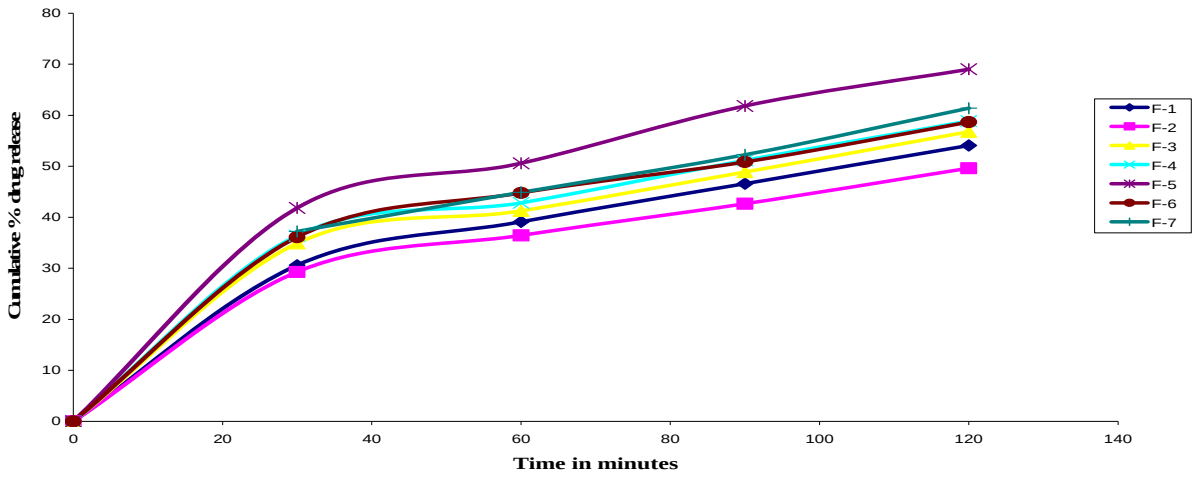
Formulation	Weight per sq.cm (gms)	Thickness (mm)	Surface pH	Folding Endurance (No. Of folds)	Fluconazole in one square cm of ocular film (μg)
F-1	0.011±0.001	0.056±0.002	6.7±0.117	262±3	6025±0.005
F-2	0.012±0.001	0.06±0.004	6.6±0.188	286±5	6450±0.006
F-3	0.009±0.003	0.06±0.002	6.6±0.125	345±5	5950±0.005
F-4	0.010±0.002	0.59±0.002	5.5±0.122	358±3	6200±0.006
F-5	0.011±0.001	0.06±0.001	6.8±0.113	385±5	5450±0.008
F-6	0.01±0.001	0.059±0.002	6.2±0119	293±5	6250±0.006
F-7	0.011±0.003	0.058±0.002	6.9±0.116	286±3	6125±0.005

All values were mean of triplicate ± standard deviation (S.D)

Table:-3 Percentage Cumulative Release Profile of F-1 to F7

Formulation	Cumulative % drug release over time period of			
	30 min	60 min	90 min	120 min
F-1	30.62 ± 1.25	39.11±0.75	46.58±0.85	54.09±1.25
F-2	29.3±0.85	36.44±0.68	42.67±0.89	49.62±1.35
F-3	34.90±0.89	41.26±0.65	48.87±0.74	56.77±0.85
F-4	36.40±1.36	42.83±1.56	51.16±0.98	58.88±0.86
F-5	41.83±0.56	50.60±1.25	61.81±0.95	69.02±0.92
F-6	36.11±0.98	44.78±0.58	50.84±1.25	58.67±0.96
F-7	37.22±0.98	44.91±1.25	52.25±0.85	61.38±0.95

Fig-1 Percentage Cumulative Release Profile of F-1 to F7



Evaluation of F-1 to F-7.

From the result it is evident all the films show uniformity in weight and thickness.For F-1 to F-7 the maximum weight per square cm observed was 0.012 ± 0.001 gm for F-5 and minimum observed was 0.009 ± 0 .003 gm for F-3.All other formulations were between these two weights.

For F-1 to F-7 the maximum thickness observed was 0.06±0.003 mm for F-5 and minimum observed 0.056 ± 0.002 mm for F-1. All formulations were between these two values of thickness.

For F-1 to F-7 the maximum folding endurance observed was 386 ± 6 for F-5 nd minimum of observed 262 ± 3 for F-1 .All other formulations were between these two values of folding endurance. Folding endurance was performed to evaluate elasticity and plasticity of films .The films containing PVA and PVP (K-30) showed maximum endurance which may be due to their continuous polymeric structure which cannot be broken easily.

For F-1 to F-7, the maximum surface pH observed was 6.9 ± 0.116 for F-7 and minimum observed was 6.3 ± 0.112 for F-4. All other formulations were between these two surface pH. The surface pH of films is important because the films are to be placed in the sensitive region of eye. Highly acidic and highly alkaline substances cause irritation and damage. Therefore the observed pH which was in neutral range shows the suitability of films to be used for ophthalmic application.

For F-1 to F-7the maximum fluconazole content observed was 6450 ± 0.006 µg /sq.cm for F-2 and minimum observed is 5025 ± 0.009 µg /sq.cm for F-3. All other formulations were between these fluconazole content. Fluconazole content showed uniformity with in the film. The variation in different films may be due to variation of thickness of films.

The most important test for drug release evaluation is *in-vitro* transcorneal permeation study. It was studied using goat cornea in a Franz diffusion cell. The receptor compartment was filled with STF (15 ml) .The total permeation study executed for 120 min.The ocular films which showed maximum fluconazole release was F-5 - 69.02 % (PVA :PVP K-30 4:2.5) and the film showed minimum was F-2- 44.45 % (PVA 5%) after 120 min. Data is shown in Table -3

The ocular films of formulation F-5 which showed appreciable result in the entire evaluation tests was subjected to *in vitro* antifungal activity testing by filter paper disc method against *Candida albicans.* The screening results a mean of 25mm zone of inhibition indicate that the ocular films of formulation F-5 exhibited antifungal activity. The ocular films of formulation F-5 has a pH which is near neutral (7.2 ± 0.113) and transcorneal permeation rate of 41.83±0.56% after 30 min and 69.02± 0.92% after 120 min .Therefore the film containing PVA:PVP (K-30) 4:2.5 was the best among the different formulations. Therefore, analysis of the results clearly shows that formulation F-5 was the most suitable for extending to further study.

Conclusion

Fluconazole is anti-fungal agent it is widely used for treating of fungal infection caused by susceptible strains of *Candida,* Tinea corporis, Tinea cruris or Tinea pedis, Onychomycosis and Cryptococcal meningitis. For ophthalmic application, fluconazole is available only in the form of eye drops. Ocular films of Fluconazole are not available in the market. Literature reports substantiate that ocular film preparation exhibits better therapeutic action when compared with the conventional ocular preparations. Hence an attempt has been made to develop an ocular film dosage form. Totally seven formulations were prepared and *in-vitro* evaluation was carried out to find out the best formulation amongst the prepared formulations, exhibiting suitable parameters.

On the basis of results of weight variation, thickness, surface pH, folding endurance, fluconazole content, F-5 was found to be the best formulation. It was concluded that fluconazole in PVA: PVP (K-30) 4:2.5 ratio would act as a suitable ocular drug delivery system than the conventional formulations.

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