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Fluconazole Ocuserts: Formulation and Evaluation

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Abstract

In the present study, it was aim to formulate ocuserts of Fluconazole and to evaluate both physicochemical parameters of *in vitro* release and *in vivo* permeation. Several polymeric systems 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 inserts were prepared by using Poly vinyl alcohol and Hydroxy propyl methyl celluloseas film forming polymers and Propylene glycol as plasticizer. Total six formulations were prepared by solvent casting technique and characterized thickness, weight variation, drug content, moisture loss, moisture absorption, folding endurance, surface pH, ocular irritation study, *in vitro* and *in vivo* release studies. The *in vitro* release studies were carried out by putting excised goat cornea between donor and receptor compartment of Franz diffusion cell. Formulation F5 shows a maximum cumulative percentage drug release of 69.02 % at the end of 2 hours through excised goat cornea. *In vivo* release profile indicated that drug release was less compared to *in vitro* release, and there was complete absence of eye irritation and redness of the rabbit eye. It can be concluded that Hydroxy Propyl methyl cellulose is a good film forming hydrophilic polymer and is a promising agent for ocular delivery.

Keywords: Film forming polymers, *In- vitro* drug release, Ocular inserts and Solvent casting technique

INTRODUCTION

Fluconazole, a synthetic antifungal agent, is a triazole derivative used in the treatment of a wide range of fungal infections and it belongs to class II of biopharmaceutical classification system (BCS) having low water solubility [1]. 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 [2]. Ocular therapy in the fungal infections would be significantly improves if the precorneal residence time of drugs could be increased [3]. Successful results have been obtained with inserts and collagen shields [4]. Several polymeric systems are investigated to fabricate ocular inserts for better ocular bioavailability and retention of drugs [5].

In the present study, it was aim to prepare and evaluate ocular films containing fluconazole along with film forming polymers namely; Poly vinyl alcohol and Hydroxy propyl methyl cellulose at different concentrations with better bioavailability and longer duration of action.

MATERIALS

Fluconazole was procured from Hetero Drugs, Hyderabad, Polyvinyl Alcohol, Poly vinyl pyrolidine K30, HPMC K-100 and Propylene Glycol were purchased from S.D. Fine Chem. Ltd, Mumbai. All other chemicals were pharmaceutical grade and used without further modification.

EXPERIMENTAL METHODS

Solubility Analysis

Pre-formulation solubility analysis was done, which included the selection of suitable solvent system to dissolve the drug as well as various excipients.

Melting Point Determination

Melting point determination of the obtained drug sample was done; as it is a first indication of purity of the sample. The presence of relatively small amount of impurity can be detected by lowering as well as widening in the melting point range.

Identification of Pure Drug

FTIR spectroscopy was used for identification of pure drug.

Determination of λ_{max}

An accurately weighed 10 mg of Fluconazole was transferred in a 100 ml volumetric flask. To the flask stimulated tear fluid was added in small proportion so as to dissolve fluconazole. The volume was made up to 100 ml with stimulated tear fluid (STF) pH 7.4 to get a concentration of 100µg/ml. 20 µg/ml solution of Fluconazole was prepared in dilution. The resulting solution was scanned in UV-Vis spectrophotometer from 400- 200 nm to determine the λ_{max}.

Construction of calibration curve

Weigh accurately 100 mg of Flucoazole& transfer into 100 ml volumetric flask & make up the final volume with pH 7.4 STF. From the stock solution different concentrations (1 - 10 µg/ml) were prepared by transferring suitable volume into the 10 ml volumetric flasks. Each concentration sample was taken & the absorption was measured at 260 nm by using UV spectrophotometer by using pH 7.4 STF as a blank. The graph was plotted by taking concentration versus absorption and the plot appeared as a straight line, the linearity was determined by using y= mx +c formula.

Compatibility studies

The compatibility of drug with the excipient used was studied by Fourier transform infrared (FTIR) spectroscopy. The FTIR spectrums of Fluconazole and Formulation (F-5) blend were studied by using FTIR spectrophotometer (Brukers) using the KBr disk method. The scanning range was 500 to 4000 cm−1, and the resolution was 1 cm−1. This spectral analysis was employed to check the compatibility of drugs with the polymers used.

Preparation of Ocuserts

The blank polymeric patches were prepared using PVA and HPMC alone by solvent casting technique [6]. Formulation of ocuserts was shown in Table 1. The polymeric drug reservoir films were prepared by dissolving 3, 4, and 5.0 % of PVA in 1/3rd volume of double distilled water. Along with this 300 mg of Fluconazole was separately dissolved in remaining amount of water and then it was poured to the polymeric solution. The solution was stirred using magnetic stirrer at 100 rpm. Then propylene glycol (6 % w/w) was incorporated to the above solution under same stirring conditions. After complete mixing the solution was cast in Petri dish (previously lubricated with Glycerin) using a ring of 5.0 cm diameter and with a funnel inverted on the surface (for uniform evaporation of solvent). The cast solution was allowed to evaporate by placing it inside a hot air oven maintained at 37 ± 2◦C, 30 ± 0.5% of RH for 24 hours. After drying the medicated films of 1 cm^2 diameter each containing 15 mg of drug were cut using a stainless steel borer, which is previously sterilized. Similar procedure was carried out for the preparation of HPMC patches.

EVALUATION PARAMETERS

Physical Characterization

The ocuserts were evaluated for their physical characters such as shape, colour, texture, and appearance.

Thickness

Ocuserts were evaluated for the thickness using a verniercaliper (Forbro Engineers, Mumbai, India) a least count of 0.01mm at different spots of the patches. The average of 6 readings was taken at different points and the mean thickness was calculated . The standard deviations (SDs) in thickness were computed from the mean value.

Weight variation

Six ocuserts were taken from each batch and their individual weights were determined by using adigital electronic balance of Shimadzu CorporationLtd, Japan [5]. The mean and standard deviation (S.D) were then calculated.

Moisture Uptake

The percentage moisture uptake test was carriedout to check physical stability or integrity of ocuserts. Ocuserts were weighed and placed in a dessicator containing 100ml of saturated solution of Aluminum chloride by which a humidity of 79.5% RH was maintained. After three days the ocuserts were taken out and reweighed, the percentage moisture uptake was calculated by using formula [7].

$$\% \text{ Moisture Uptake} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

Moisture Loss

The percentage moisture loss was carried out to check integrity of the ocuserts at dry condition. Ocuserts were weighed and kept in a desiccator containing anhydrous Calcium chloride. After 3 days, the ocuserts were taken out and reweighed; the percentage moisture loss was calculated using the formula.

$$\% \text{ Moisture Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Folding Endurance

The folding endurance is expressed as the number of times the insert is folded at the same place, either to break the specimen or to develop visible cracks [7]. 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.

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 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.

Uniformity of fluconazole content

The ocuserts were placed in 5ml of pH 7.4 STF and were shaken in orbital shaker incubator at 50 rpm to extract the drug from ocuserts. After incubation for 24 h, the solution was filtered through a 0.45 μm filter and the filtrate was suitably diluted with STF solution. The absorbance of the resulting solution was measured at 260 nm [7].

In Vitro transcorneal permeation Studies

Whole eyeball of goat was transported from local butcher shop and cornea was carefully isolated along with 2 to 4 mm of surrounding tissue and was washed with cold normal saline free from proteins. Isolated cornea was mounted between clamped donor and receptor compartments of Franz diffusion [8]. A strip of film (1cm) placed in donor compartment and the solution (pH 7.4 STF) in receptor compartment were stirred at 100 rpm by using magnetic stirrer and the temperature was maintained at 37^0 C$\pm5^0$ C. At 15, 30, 45, 60, 90, 120 and 150 min time intervals 0.5 ml of test sample was removed from the receptor and the volume was replaced by adding fresh buffer. The test samples were filtered and the absorbance of each sample was measured at 260 nm by using UV spectrophotometer and reagent blank (pH 7.4 STF). The absorbance was converted into concentration by using the standard curve. The release rates were calculated and the graphs were plotted taking time on X- axis & cumulative amount on Y- axis.

Ocular Irritation

The potential ocular irritation and/or damaging effects of the ocusert under test were evaluated by observing them for any redness, inflammation, or increased tear production [9]. Formulation was tested on five rabbits by placing the inserts in the cul-de-sac of the left eye. Both eyes of the rabbits under test were examined for any signs of irritation before treatment and were observed up to 12 hours.

In Vivo Drug Release Study

Out of six batches of formulations F-5 was taken for in vivo study on the basis of in vitro drug release studies. The ocuserts were sterilized by using UV radiation before in vivo study. After sterilization, ocuserts were transferred into polyethylene bag with the help of forceps inside the sterilization chamber itself. Albino rabbits of either sex (New-Zealand strain), weighing between 2.5–3.0 kg, were used for the experiment [10]. The ocuserts containing Fluconazole were taken for in vivo study was placed into the lower conjunctival cul-de-sac. The ocuserts were inserted into each of the six rabbits and at the same time the other eye of six rabbits served as control. Ocuserts were removed carefully at 30, 60, 90, 120, and 150 minutes and analyzed for drug content. The drug remaining was subtracted from the initial drug content of ocuserts that will give the amount of drug released in the rabbit eye.

RESULTS AND DISCUSSION

Solubility Studies

The saturation solubility of drug in distilled water and stimulated tear fluid (STF pH 7.4) was determined by adding an excess of drug to 10 ml distilled water and STF in glass stoppered flask. The stoppered flasks were rotated for 24 hr in orbital shaker at 37^oC. The saturated solutions were filtered through a 0.45 µm membrane filter, suitably diluted with water and analyzed by UV spectrophotometer. The solubility of pure drug in water and STF (pH 7.4) was found to be 27.04 µg/ml and 57.06 µg/ml.

Melting Point Determination

After performing capillary method melting point of Fluconazole found in range of 139-140^oC

Identification of Pure Drug

FT-IR spectroscopy was used to determine the functional group present in the pure drug sample.The spectrum of Fluconazole presenting the characteristic peak of triazole group C-H stretching at 2956cm-^{1}, C-F stretching at 1275.76cm-^{1}, 2.4-Difluorobenzyl group at 1619.47 cm-1 presenting C=C stretching vibration, peak at 1422.39 cm-1 was due to CH_2 stretching vibration and peak at 1140.97cm-1 was due to C·C stretching vibration. IR Spectra of Fluconazole was shown in Fig.3

Determination of λmax

The Fluconazole solution was scanned in UV-Vis spectrophotometer from 400- 200 nm to determine the λmax. The λmax was found to be at 260 nm, so the calibration curve of Fluconazole was developed at this wavelength. λmax of Fluconazole was shown in Fig.1

Construction of calibration curve

The standard curve of Fluconazole was done by using pH 7.4 STF as the medium and making the concentrations of 1 to 10 µg/ml solutions. The absorbance of solutions was examined under UV-spectrophotometer at an absorption maximum of 260 nm. The standard graph was constructed by taking the absorbance on Y-axis and concentrations on X-axis. The standard calibration curve

Table No 1: Formulation of Fluconazole Ocuserts

Ingredients(Gms)	Formulation Code					
	F1	F2	F3	F4	F5	F6
PVA	0.45	0.60	0.75	--	--	--
HPMC	--	--	--	0.45	0.60	0.75
PG	0.9	0.9	0.9	0.9	0.9	0.9
WATER	15	15	15	15	15	15
DRUG	0.30	0.30	0.30	0.30	0.30	0.30

of Fluconazole in pH 7.4 STF was shown in Table 2 and Figure 2. Drug concentration and absorbance followed linear relationship the curve obeyed Beer-Lambert's law and the correlation coefficient value (R^2) is 0.998.

Compatibility Studies

FTIR techniques have been used here to study the physical and chemical interaction between drug and excipients used. For fluconazole 2956 cm-1 C-H stretching, 1275 cm-1 C-F stretching, 1445 cm-1 C-H bending, For HPMC & Fluconazole 2956 cm-1 C-H stretching, 1446 cm-1 C-H bending C-F bond is not found as shown in Fig. 4, For PVA & Fluconazole 2956 cm-1 C-H Stretching-g Ar, 1445 cm-1 C-C multiple bond stretching (Aromatic) as shown in Fig. 5, which show there were no physical interactions because of some bond formation between drug and polymers.

Preparation of Ocuserts

The ocuserts were prepared by solvent casting method and were cut into 1cm^2 strips. Each strip contains 15 mg of fluconazole. Totally six formulations were prepared and named as F-1 to F-6. The formulation F-1 to F-3contains only PVA, F-4 to F-6 contain HPMC (K-100). The polymers PVA and HPMC used as film forming agents, Propylene glycol used for a plasticizing effect, glycerine used as lubricant and water used as medium for preparation. Fluconazole dosage fixed at 300 mg and the polymers PVA and HPMC used in concentration range of 3, 4 and 5%w/v. The prepared ocuserts were shown in Figure 6.

EVALUATION OF OCUSERTS

Physical Characterization

The prepared fluconazole ocuserts were white in colour and has smooth surface.

Thickness

Thicknesses of the ocuserts were found to be directly related to the concentration of the polymers. Thickness of the ocuserts varied between 0.315±0.09 mm to 0.380±0.02 mm. The result showed that thickness was uniform and ocuserts were not thick enough to produce any irritation while placing and being in *cul-de-sac.*

Weight variation:

The weight of formulations was determined by digital electronic balance. The result showed that weights of formulations were ranging from 0.022±0.031gm. to 0.036 ±0.079gm. This indicates that there was no significant weight variation in all formulations. The data is shown in Table 4.

Moisture Uptake:

Among the formulations tested F6 and F1 shows the maximum and minimum moisture uptake i.e., 9.84 ± 0.058 and 4.78 ± 0.222 respectively. The maximum moisture uptake from ocusert may be due to the high concentration of hydrophilic polymer HPMC, which readily absorbs moisture when exposed to atmosphere.

Moisture Loss:

Among the formulations tested F6 and F1 shows maximum and minimum moisture loss i.e. 9.2 ± 0.254 and 6.2 ± 0.574 respectively. Data is shown in Table 5. The minimum moisture loss shown by the formulation F1 was mainly due to the PVA as rate controlling membrane, which retain the moisture within the matrix.

Table No 2: Standard Calibration Curve of Fluconazole

Concentration (µg/ml)	Absorbance in pH 7.4 STF (nm)
0	0.00
1	0.155 ± 0.003
2	0.257 ± 0.002
3	0.373 ± 0.001
4	0.474 ± 0.001
5	0.572 ± 0.002
6	0.684 ± 0.004
7	0.773 ± 0.006
8	0.882 ± 0.006
9	0.982 ± 0.007
10	1.097 ± 0.008

Table No 3: Thickness of Patch

Formulation	Thickness (mm)
F1	0.315±0.09
F2	0.320±0.07
F3	0.328±0.06
F4	0.370±0.06
F5	0.375±0.04
F6	0.380±0.02

Mean ± S.D. of six determinations

Table No 4: Weight Variation of Patch

Formulation	Weight Variation(gm)
F1	0.022±0.091
F2	0.024± 0.052
F3	0.027± 0.073
F4	0.019±0.031
F5	0.030±0.042
F6	0.036±0.079

Mean ± S.D. of six determinations

Table No 5: Moisture Uptake & Moisture Loss

Formulation	*Moisture Uptake*	*Moisture Loss*
F1	4.78 • ±0.222	6.2 ± 0.574
F2	5.35 • ±0.155	7.1 ± 0.324
F3	6.28 • ±0.169	7.3 ± 0.125
F4	7.51 • ±0.153	8.6 ± 0.261
F5	8.15 • ±0.048	9.1 ± 0.564
F6	9.84 • ±0.058	9.2 ± 0.254

Mean ± S.D. of six determinations

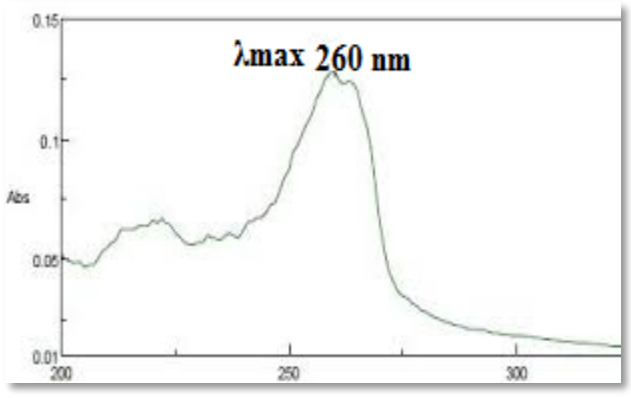

Figure 1: UV Spectra of Fluconazole

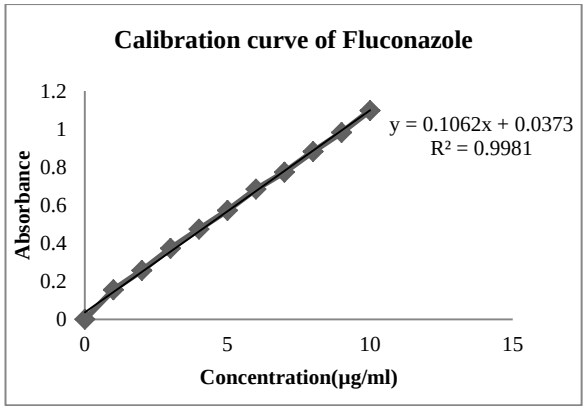

Figure 2: Standard Calibration Curve of Fluconazole in pH 7.4 STF

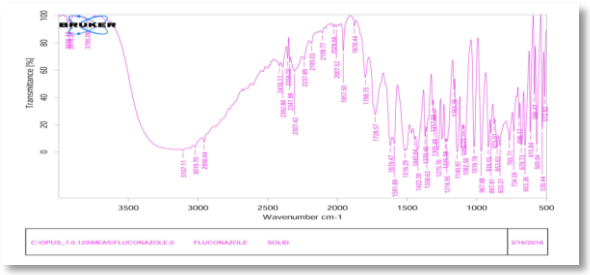

Figure 3: IR Spectra of Pure Drug Fluconazole

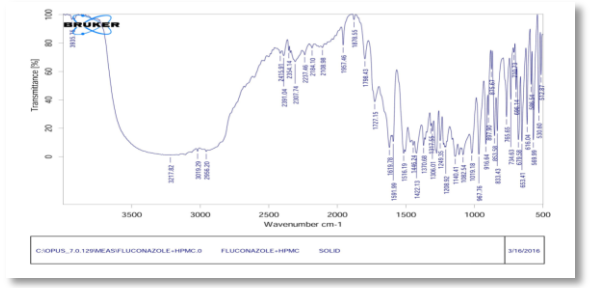

Figure 4: IR Spectra of Fluconazole + HPMC

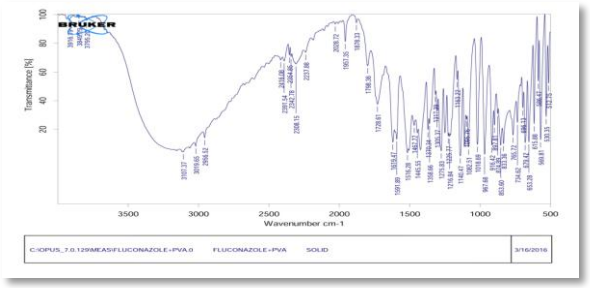

Figure 5: IR Spectra of Fluconazole + PVA

Folding Endurance

The folding endurance is expressed as the number of folds. This test is important to check the ability of the sample to withstand folding. This also gives an indication of brittleness. The folding endurance for all formulations was good. The maximum folding endurance for F3 ophthalmic insert was 293±3 folding which may be due to presence of PVA and formulation F4 showed minimum folding endurance 261±5 folding. Data is shown in Table 6. The inserts containing PVA showed maximum endurance which may be due to their continuous polymeric structure which cannot be broken easily.

Surface pH Determination

The surface pH of inserts is important because the inserts are to be placed in the sensitive region of eye. Highly acidic and highly alkaline substances cause irritation and damage. The maximum surface pH observed was 6.9 ± 0.116 for F-6 and minimum observed was 6.3 ± 0.117 for F-1. Therefore the observed pH which was in neutral range shows the suitability of inserts to be used for ophthalmic application. Data is shown in Table 7.

Uniformity of fluconazole content

The drug content uniformity of all formulations was in the range of 89.51%± 0.053 to 95.21% ± 0.046. Formulation F-1 & F-4 showed the least drug content and formulation F-5 showed the highest drug content. Data is shown in Table 8. This indicates the method used for preparing ocusert was reliable. This variation may be due to variation of thickness of inserts.

***In Vitro* Transcorneal Permeation Studies**

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 150 min. The ocular films which showed maximum fluconazole release was F-5 - 65.23 % (HPMC 4%) and the film showed minimum was F-1- 42.25 % (PVA 3%) after 150 min. Data is shown in Table 9 and Figure 7.

Ocular Irritation

The formulation F5 insert was placed in cul-de-sac of the rabbit eye and observed visually for any irritant effect. No sign of redness, inflammation, or increased tear production were observed. Thus, results of the test have shown that there were no signs of irritation observed.

***In Vivo* Drug Release Study**

The ocular inserts of formulation F-5 which showed appreciable result in drug content uniformity and *in-vitro* release studies was subjected to in vivo drug release study. The *in vivo* cumulative percent drug release from F-5 was found to be 52.14% after150 minutes respectively, which is found to be less when compared to *in vitro* drug release studies. The *in vivo* cumulative drug release versus time for optimized formulation was showed in Table 10 and Figure 8.

Table No 6: Folding Endurance

Formulation	No. of Foldings
F1	262±3
F2	263±5
F3	293±3
F4	261±5
F5	286±3
F6	265±5

Mean ± S.D. of six determinations

Table No 7: Surface pH of the films

Formulation	Surface pH
F1	6.3±0.117
F2	6.6±0.188
F3	6.6±0119
F4	6.8±0.113
F5	6.6±0.125
F6	6.9±0.116

Mean ± S.D. of six determinations

Table No 8: Uniformity of fluconazole content

Formulation	Drug Content	
	In mg	In %
F1	13.42± 0.221	89.51±0.053
F2	13.67± 0.311	91.16±0.024
F3	13.59± 0.546	90.65±0.022
F4	13.42±0.325	89.51 ±0.021
F5	14.28±0.521	95.21±0.046
F6	13.24± 0.322	88.32±0.029

Mean ± S.D. of six determinations

Table No 9: Cumulative percentrelease of fluconazole

Time (min)	F1	F2	F3	F4	F5	F6
15	15.43±0.52	16.39±0.53	19.32±0.21	16.49±0.36	18.52±0.32	14.19±0.23
30	18.52±0.72	21.92±0.26	29.32±0.54	22.59±0.66	29.27±0.53	20.48±0.56
45	22.45±0.66	29.19±0.63	36.86±0.19	35.48±0.35	39.37±0.62	40.17±0.67
60	28.28±0.64	37.23±0.81	42.23±0.28	41.80±0.58	47.14±0.81	45.82±0.58
90	32.20±0.32	42.35±0.76	50.48±0.36	46.46±0.52	56.71±0.78	50.14±0.47
120	37.80±0.71	49.21±0.46	58.12±0.59	51.15±0.24	61.04±0.24	61.36±0.54
150	42.25±0.34	54.62±0.52	60.46±0.83	58.23±0.38	65.23±0.59	63.49±0.69

Table No 10: In vivo cumulative percent drug **release**

Time(min)	*In vivo* cumulative drug release from F5
30	14.19±0.32
60	31.17±0.76
90	39.82±0.58
120	45.14±0.33
150	52.14±0.54

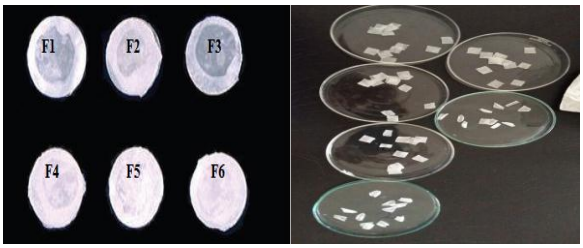

Figure 6: Preparation of Fluconazole Ocuserts

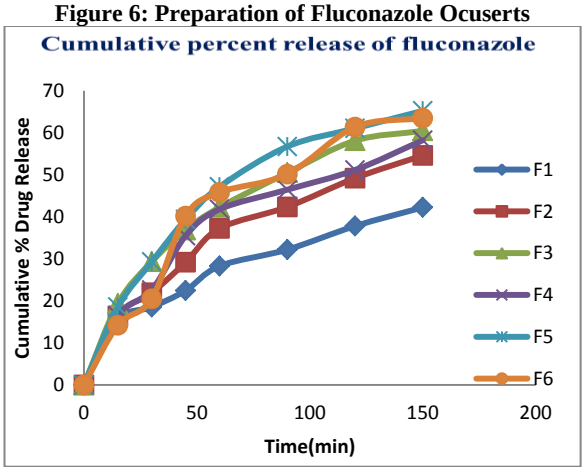

Figure 7: Cumulative percentrelease of fluconazole

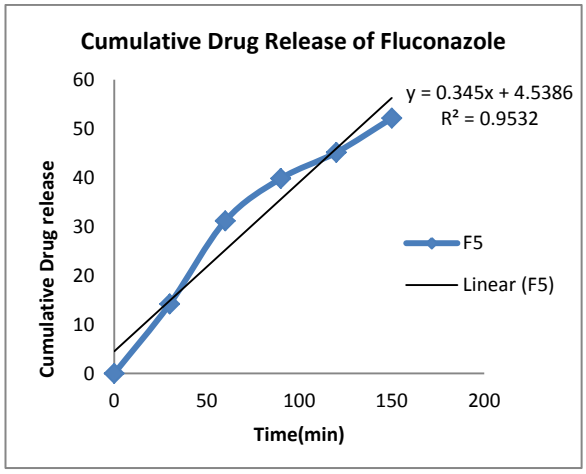

Figure 8: In vivo cumulative percent drug **release**

CONCLUSION

The experimental findings concluded that Hydroxy Propyl methyl cellulose is a good film forming hydrophilic polymer and is a promising agent for ocular delivery. Incorporation of propylene glycol enhances the permeability and thus therapeutic levels of the drug could be achieved. *In vivo* release profile indicated that drug release was less compared to *in vitro* release, and there was complete absence of eye irritation and redness of the rabbit eye. Further future work will be progressed to establish the therapeutic utility of these systems by pharmacokinetic and pharmacodynamic studies in human beings.

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