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FORMULATION AND EVALUATION OF FLUCONAZOLE OCULAR INSERTS

***V.Viswanath, B. Narasimharao, M.Purushothaman, G. Sowjanya, S.Sreevani**

Asst. Professor of PRRM College of Pharmacy, Kadapa,516003, Andhra Pradesh, India.

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***Correspondence for Author**
V.Viswanath
Asst. Professor of PRRM College of Pharmacy, Kadapa,516003, Andhra Pradesh, India.

ABSTRACT

An attempt has been made to formulate sustained release ocular inserts of Fluconazole for the treatment of fungal infections using Poly vinyl alcohol, PVPK-30, Hydroxy, propyl methyl cellulose as polymer and glycerin, propylene glycol as plasticizers by Mercury Substrate method. The aim of this study is to increase the contact time of Fluconazole to achieve sustained release, reduction in frequency of administration, improving patient compliance, and greater therapeutic efficacy. IR spectral studies were performed to confirm the interaction of drug with polymers using KBr disc method. The prepared ocular inserts were then evaluated for uniformity of thickness, weight variation, drug content, surface pH, swelling index, folding endurance, percentage moisture loss, and moisture absorption etc. *In vitro* diffusion studies of formulated ocular inserts were performed by studying diffusion through dialysis membrane using STF pH 7.4 as a medium. The films were uniform in weight, thickness, and visually smooth surfaced. Among the all formulations, formulation (F11) containing HPMC, PG, Glycerin showed complete sustained release of drug with 95.24 % at the end of 12 hours. The drug release from the film follows zero order kinetics and non-fickian diffusion mechanism.

KEYWORDS: Fluconazole ocular inserts, Mercury substrate method, Zero order release, In vitro drug release.

INTRODUCTION

The eye is one of the most highly structured organ in human beings. The structures contained in the eye were developed to receive and transduce signals from visible light. Ocular drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical

scientists. This drug delivery is normally used for the local therapy against systemic therapy, to avoid risk of eye damage from high drug concentrations in the blood. The anatomy, physiology and biochemistry of eye turn into this organ is highly impervious to foreign substances, so a significant challenge to the formulator to circumvent the protective barriers of eye without causing permanent tissue damage.[1,2]

The conventional dosage forms of the ocular drug delivery system like eye drops, eye suspensions, eye ointments and solutions[3] are easily instilled, but the main drawback of this dosage form is that it produces poor bioavailability and therapeutic response due to rapid pre corneal elimination of the drug. This conventional dosage forms may also produce poor patient compliance because the patient need to take dose several times in a day in order to achieve the therapeutic response.[4,5]

Because of poor ocular bioavailability, the frequent periodic instillation of eye drops becomes necessary to maintain a continuous sustained level of medication. It gives the eye massive and unpredictable dose of medication, unfortunately a higher drug concentrations in the eye this causes both ocular and systemic side effects. In order to overcome this drawback a newer approaches for ocular drug delivery systems are being explored to develop extended duration and controlled release strategy. The large number of eye infections are caused by bacteria. In addition to that virus, fungus and protozoan also cause eye infections. The most commonly occurred eye infections are[6,7,8] Conjunctivitis, Blepharitis, Keratitis, Cataract, Iritis (anterior uveitis), Glaucoma.

Absorption of drugs in eye[9]

Topical delivery into the cul-de-sac is, by far, the most common route of ocular drug delivery, absorption from this site by Corneal, Non-corneal routes. The maximum absorption of drugs takes place through cornea, which leads the drug into aqueous humor. The non-corneal route involves absorption of drugs across the sclera and conjunctiva into the intraocular tissues. This route is not productive as it restrains the entry of drug into the intraocular tissues. This non-corneal route of administration may be significant for drug molecules with poor corneal permeability.

Ocular inserts[10] are defined as sterile preparations, with a solid or semisolid consistency and whose size and shape are especially designed for ophthalmic application. The main objective

of the ocular inserts is to increase the contact time between the preparation and the conjunctival tissue to ensure a sustained release.

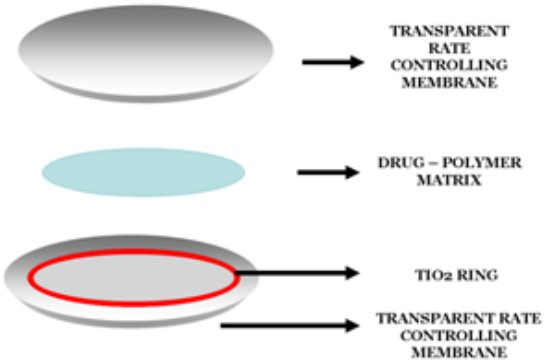

Figure No.1: Keratitis. Figure No.2: Schematic representation of ocular inserts.

These ocular inserts are mainly overcome the disadvantages of conventional dosage forms of the ocular drug delivery like eye drops, eye ointments, eye suspensions and solutions. Ocular inserts maintain the drug concentration within a desired range.

In the recent years, there has been explosion of interest in the polymer based delivery devices, adding further dimension to topical drug delivery thereby promoting the use of polymers such as collagen and fibrin fabricated into erodible inserts for placement in cul-de-sac. Utilization of the principles of controlled release as embodied by ocular inserts offers and attractive approach to the problem of prolonging precorneal drug residence times. Ocular inserts also offer the potential advantage of improving patient compliance by reducing the dosing frequency.

MATERIALS AND METHODS

Fluconazole obtained from Yarrow chemical product, Mumbai, PVA obtained from Finar chemicals Ltd, Ahmadabad, PVP K-30 from Finar chemicals Ltd, Ahmadabad, HPMC from Finar chemical limited, Ahmadabad, Propylene glycol from Finar chemical limited, Ahmadabad, Glycerin from Finar chemical limited, Ahmadabad.

Method of preparation of ocular inserts

The Ocular inserts are prepared by using Mercury substrate method. In this method the polymers and Fluconazole were weighed in requisite ratios and it is made up to 100ml with water. The above solution was sonicated in an ultrasonicator at a speed of 80 MHz for 20 min. After dispersion the different volumes of propylene glycol, glycerin was added as a

plasticizer. This is allowed to stand overnight to remove entrapped air bubbles. The solution was poured on the mercury placed in a glass petridish and dried at room temperature for 24hrs. During drying, the glass petridish was covered with inverted funnel to ensure slow evaporation. After drying, the dried ocular films were carefully removed and cut into square films with the sterilized needle.

Table No.1: Composition of Ocular inserts

Formulation Code	PVA (gm)	PVP K-30 (gm)	HPMC (gm)	Propylene Glycol(ml)	Glycerin (gm)	Fluconazole (gm)	Water q.s
F1	1	-	-	0.04	-	0.3	100
F2	1	-	-	0.05	-	0.3	100
F3	1	-	-	0.06	-	0.3	100
F4	1	-	-	0.07	-	0.3	100
F5	1	-	-	0.08	-	0.3	100
F6	1	0.3	-	0.02	-	0.3	100
F7	1	0.4	-	0.04	-	0.3	100
F8	1	0.5	-	0.06	-	0.3	100
F9	-	-	0.5	0.04	0.5	0.3	100
F10	-	-	0.5	0.06	0.5	0.3	100
F11	-	-	0.5	0.08	1	0.3	100

Preformulation studies

Preformulation testing is the first step in the rationale development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable, efficacious and safe dosage form. Hence preformulation studies were carried out on the obtained samples of drug for identification and compatibility studies.

1. Identification of Drug: The obtained sample was examined by infrared absorption spectral analysis and was compared with the reference standard IR spectrum of Fluconazole.

Method: IR Spectra of drug and drug-excipient blends were recorded on an IR spectrophotometer (Shimadzu Corporation, Japan) in the range of 4000-400 Cm^{-1} using potassium bromide discs.

2. Determination of Melting point: Melting point of Fluconazole was determined by open capillary method. Melting point apparatus is most often used for the determination of the

melting point of a solid. A few crystals of the compound are placed in a thin walled capillary tune 10-15 cm long, about 1mm in inside diameter, and closed at one end.

3. Determination of solubility: The known excess amount of drug was added to 5 ml of water, methanol, DCM and these samples were rotated at 20 rpm in a water bath (37±0.50C) for 2hrs.The samples were then filtered through 0.45 µm membrane filter, suitably diluted, and analyzed visually.

4. Compatibility Studies: The compatibility of drug and polymers under experimental condition is important prerequisite before formulation. Incompatibility between drugs and excipients can alter stability and bioavailability of drugs, thereby, affecting its safety and/or efficacy. Drug-excipient compatibility testing at an early stage helps in the selection of excipients that increases the probability of developing a stable dosage form. The compatibility between pure drug, Polymers and excipients were detected by FTIR spectra obtained on Brucker, Germany. For the preparation of KBr pellets, the solid powder sample were ground together in a mortar with 5 times quantity of KBr the finely grounded powder was introduced into a stainless steel die. The powder was pressed in the die between polished steel anvils at a pressure of about 50 pounds. The spectras were recorded over the wave number of 4000^{-1} to $400cm^{-1}$.

Evaluation of ocular inserts

1.Uniformity of Thickness: Insert thickness was measured by a Vernier Caliper at five different points on the film. The mean thickness was calculated.

2.Uniformity of weight: The films were taken from each batch and their individual weights were determined by using electronic balance. Mean weight of inserts of each formulation was calculated.

3.Folding Endurance: Folding Endurance of the film was determined by repeatedly folding the inserts at the same place till it breaks. The ocular inserts was folded in the center, between finger and thumb and then opened. This process was repeated for a number of times, until the film breaks and the number of folds were recorded.

4. Uniformity of Drug Content: Uniformity of drug Content was determined by assaying the individual inserts. Each insert is dissolved in 100ml of STF in a beaker and filtered. Take 10ml of the above solution and it is diluted to 100ml with STF. From this solution take 1ml

and diluted to 10ml with STF. The absorbance of these solutions was then measured on UV-Visible spectrophotometer at 260nm.

5. Swelling Index: The three films were weighed and placed separately in beakers containing 4ml STF. After a period of 5 minutes, the films were removed and the excess water on their surface was removed using a filter paper and then again weighed till there was no increase in the weight and then the swelling index was calculated.

6. Percentage Moisture Absorption: This test was carried out to check the physical stability of the ocular inserts at high humid condition. The ocular inserts were pre weighed accurately and kept in desiccators containing 100ml of saturated solution of aluminium chloride. After 72hrs, the films were taken out, weighed and % Moisture Absorption was calculated by using formula.

$$\% \textbf{ Moisture Absorption} = \frac{\textbf{Final weight} - \textbf{Initial weight}}{\text{Initial weight}} \times \textbf{100}$$

7. Percentage Moisture Loss: This test was carried out to check the integrity of ocular inserts at dry condition. The ocular inserts were weighed accurately and kept in a desiccators containing anhydrous calcium chloride. After 72hrs, the films were taken out weighed and % moisture loss was calculated by using formula.

$$\% \textbf{ Moisture loss} = \frac{\textbf{Initial weight} - \textbf{Final weight}}{\text{Initial weight}} \times \textbf{100}$$

8. Surface pH: Inserts were left to swell for 5hrs on agar plate prepared by dissolving 2% agar in warm STF of pH 7.2 under stirring and then pouring the solution into petridish till gelling at room temperature. The surface pH was measured by means of digital pH meter.

***9. In*-vitro Diffusion Studies:** The *In*-vitro diffusion of drug from the different ocular insert was studied using the classical standard cylindrical tube fabricated in the laboratory. A simple modification of open ended glass tube was used. The diffusion cell membrane (pre hydrated cellophane) was tied to end of open cylinder, which acted as a donor compartment. An ocular insert was placed inside this compartment. The diffusion cell membrane acted as corneal epithelium. The entire surface of the membrane was in contact with the receptor compartment containing 25ml of STF in 100ml beaker. The content of receptor compartment was stirred continuously using a magnetic stirrer and temperature was maintained at $37^0C \pm 0.5^0C$. At

specific interval of time, 5ml of the receptor compartment was replaced with fresh STF solution. The aliquot was analyzed for the drug content using UV-Visible Spectrophotometer at 260nm after appropriate dilutions against reference using as STF as blank.

RESULTS AND DISCUSSION

The IR spectrum of pure drug was found to be similar to the standard spectrum of Fluconazole. The spectrum of Fluconazole shows its functional groups at the frequencies shown below

Table no.2: Functional groups and Frequencies of Fluconazole

Functional groups	Frequencies
O-H stretch	3221.86
C-O stretch	1140.48
C=N stretch	1620.39
C-N stretch	1423.60
C-C stretch	1514.23
C-H Rocking	2926.16
C-F stretch	1018.10 1278.37

Compatibility studies of pure drug Fluconazole with polymers were carried out prior to the formulation of ocular inserts indicates that there was no significant change in the chemical integrity of the drug.

The melting point of Fluconazole was found to be 140^{o} C and the Solubility reveals that it was slightly soluble in water, feely soluble in methanol and soluble in dichloromethane and acetone.

Uniformity of thickness of ocular inserts was found to be in the range of 0.053mm to 0.093mm and uniformity of weight was found to be in the range of 5.1mg to 6.03mg which complied with the standards of B.P.

The folding endurance test lie between >200 to <300 and Drug content values ranged from 77.14% to 96.4 which showed reproducible results**.**Swelling index values are obtained from the range of 3.17% to 4.79% which indicates that all the formulations cause less discomfort.

Percentage moisture absorptions were observed between 3% to 8% and the Percentage moisture loss were observed from 2% to 7% which revealed that there was no change in

integrity at high humid and dry conditions. The surface pH was observed in neutral range shows that the films are suitable for ophthalmic application.

The *In*-vitro release of Fluconazole ocular inserts was performed in STF pH 7.4. *In*-vitro drug release studies had clearly indicated that F11 has sustained release because in that HPMC , Propylene glycol and Glycerin were used, in that HPMC used as a polymer and it is responsible for sustained release and Propylene glycol, Glycerin were used as a plasticizers and they give flexibility and elasticity to the inserts.

Table No.3: Invitro Drug Release Profile

s.no	Time (hrs)	% cumulative drug release										
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
1	1	17.21	18.55	19.41	16.81	12.39	12.71	18.34	16.30	15.63	17.52	19.72
2	2	22.99	25.04	25.73	21.33	18.02	17.52	22.95	24.70	19.86	22.93	25.20
3	3	28.17	30.92	31.11	26.67	23.84	23.09	28.88	30.40	27.26	28.02	30.46
4	4	34.25	37.10	37.30	33.52	34.22	29.93	34.28	35.50	32.51	32.84	37.63
5	5	41.34	44.79	44.03	39.19	39.61	36.51	39.72	41.59	38.06	39.01	44.33
6	6	47.88	52.45	50.69	45.49	45.88	43.19	45.73	47.78	43.88	45.05	51.70
7	7	54.94	59.55	57.86	52.10	52.36	51.39	52.19	54.95	50.20	53.61	60.16
8	8	61.48	68.69	66.56	60.18	59.47	63.56	58.58	61.95	56.82	62.91	67.81
9	9	68.41	76.03	74.00	68.21	71.01	71.08	65.57	72.61	63.73	71.72	76.59
10	10	76.39	83.48	82.25	78.02	79.38	82.60	74.37	83.35	72.60	82.92	85.62
11	12	84.53	90.65	89.81	87.56	88.22	91.51	84.16	92.91	82.82	94.82	95.24

Table No.4: Physical evaluations of fluconazole ocular inserts

Formulation Code	Uniformity of Thickness(mm)	Uniformity of Weight(mg)	Folding Endurance (No. of folds)	Drug Content (%)
F1	0.053	5.1	220	77.14
F2	0.059	5.23	225	82.16
F3	0.084	5.3	223	81.00
F4	0.062	5.53	228	84.8
F5	0.064	5.4	221	83.3
F6	0.075	5.46	240	88.7
F7	0.093	6.03	246	86.7
F8	0.063	5.36	248	92.5
F9	0.072	5.83	230	89.1
F10	0.083	5.63	232	94.5
F11	0.089	5.96	236	96.4

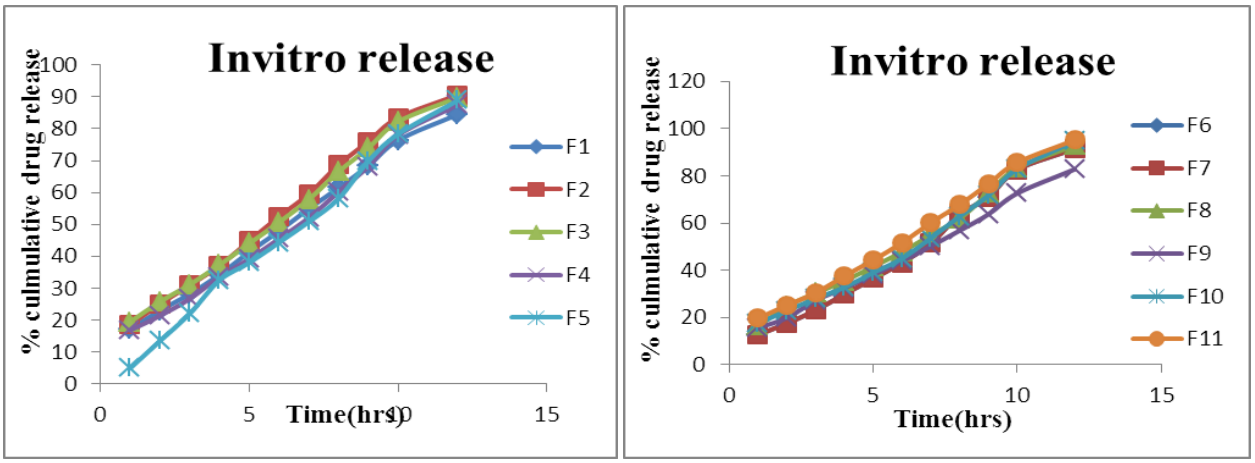

Figure No.3: Invitro drug release profile for all formulations

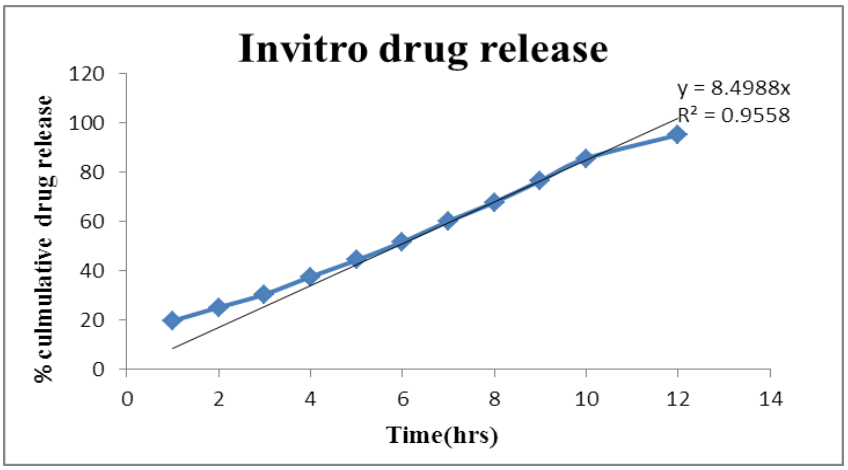

Figure No.4: Invitro drug release for best formulation (F11)

Table No.5: Physical evaluations of fluconazole ocular inserts

Formulation Code	Swelling Index	% Moisture Absorption	% Moisture Loss	Surface pH
F1	3.92	4.90	3.92	6.5
F2	4.52	3.63	2.10	5.8
F3	3.77	3.39	5.28	6.2
F4	4.27	4.33	5.42	6.1
F5	3.70	5.37	4.25	5.9
F6	5.49	6.95	6.22	6.3
F7	3.31	8.12	7.79	6.5
F8	3.17	7.83	4.10	6.8
F9	4.05	6.86	6.51	6.6
F10	4.79	5.15	4.79	6.7
F11	4.02	7.12	3.85	6.9

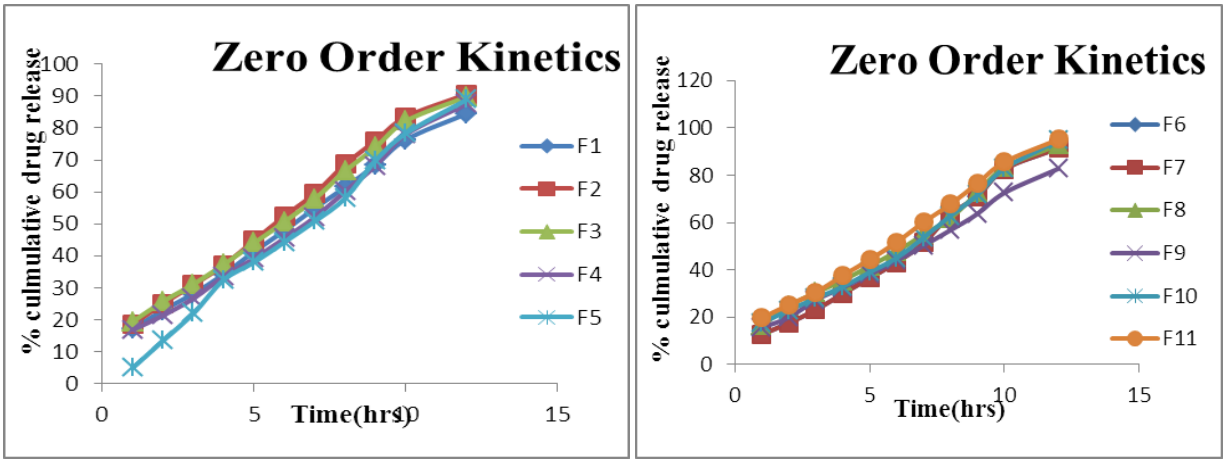

Figure No.5: Zero Order Kinetics for all formulations

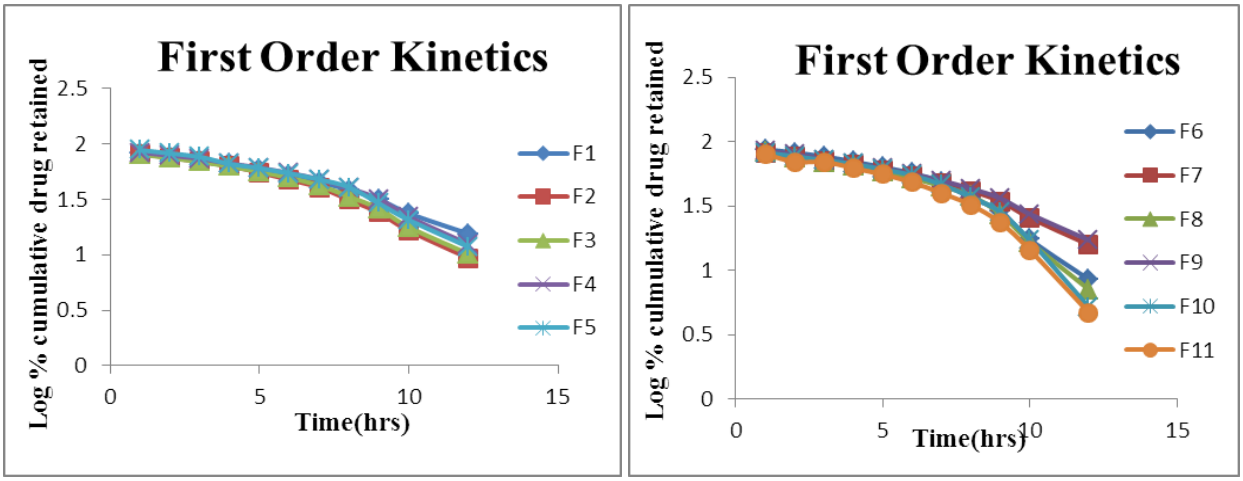

Figure No.6: First Order Kinetics for all formulations

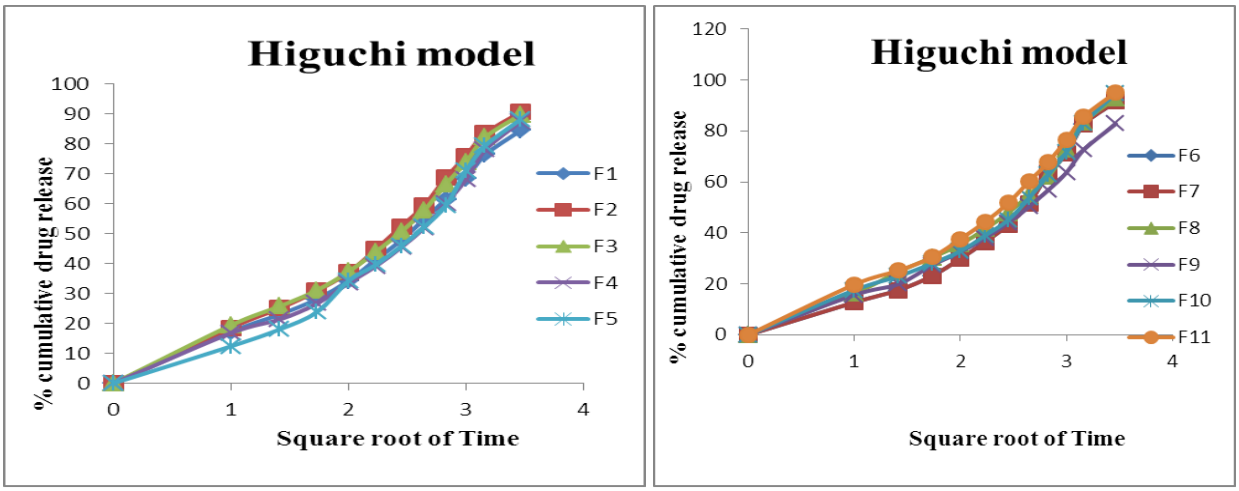

Figure No.7: Higuchi model plot for all formulations

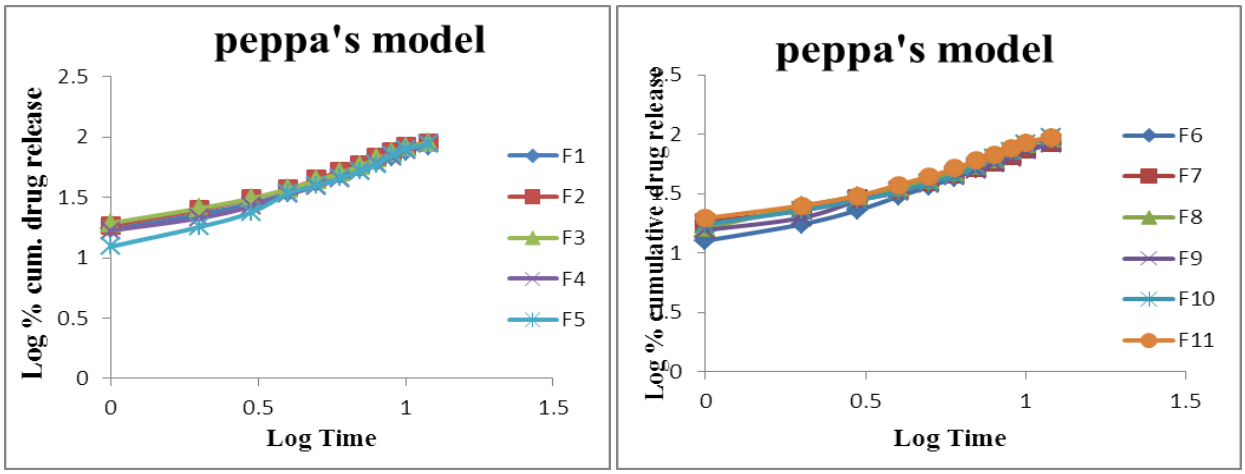

Figure No.8: Korsemeyer peppa's model for all formulations

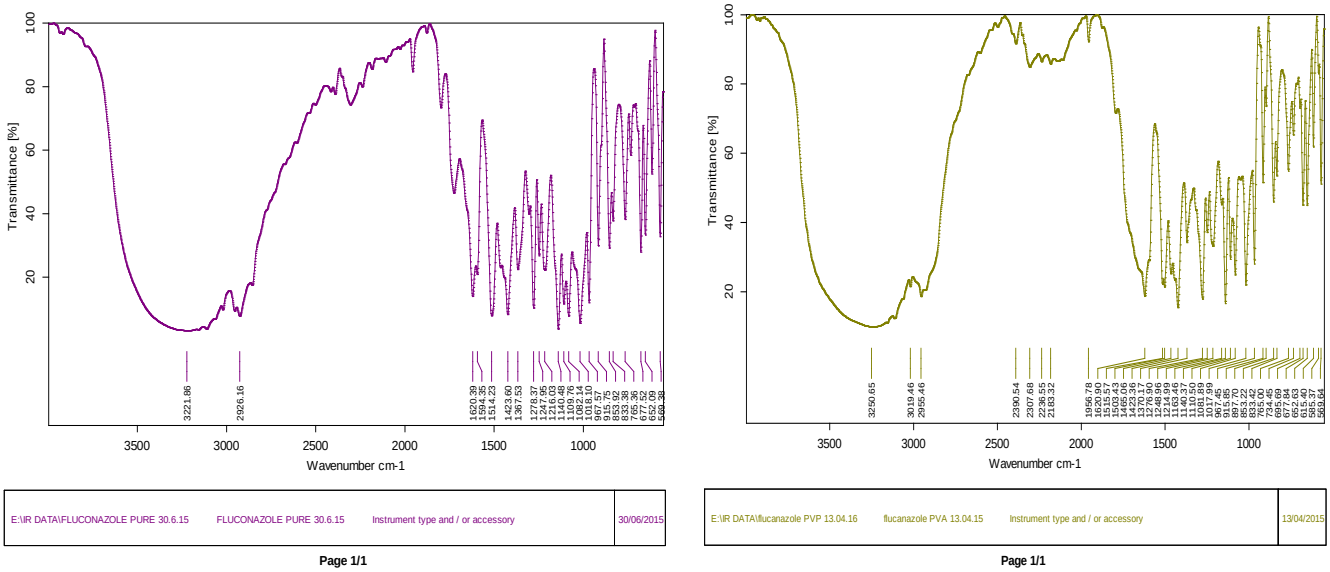

Fig no: 9FTIR for the pure drug Fluconazole Fig.no:10FTIR for drug along with PVP K-30

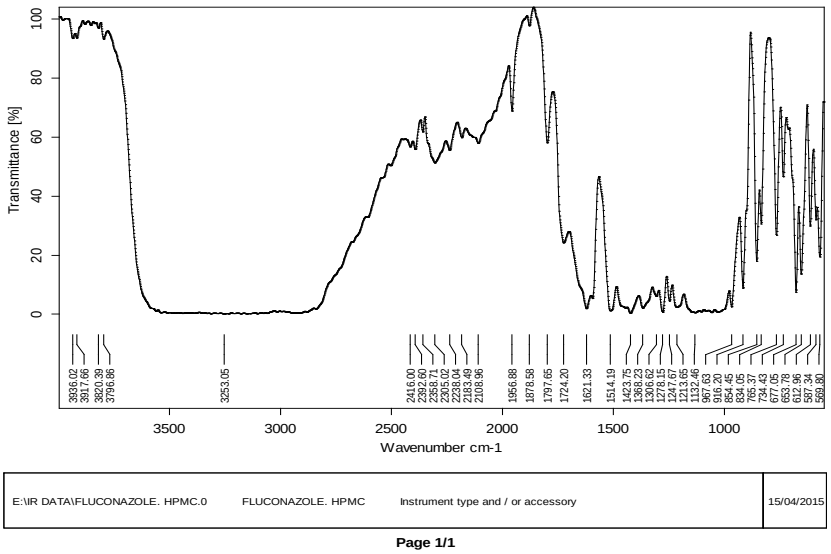

Fig. no: 11 FTIR for drug along with HPMC

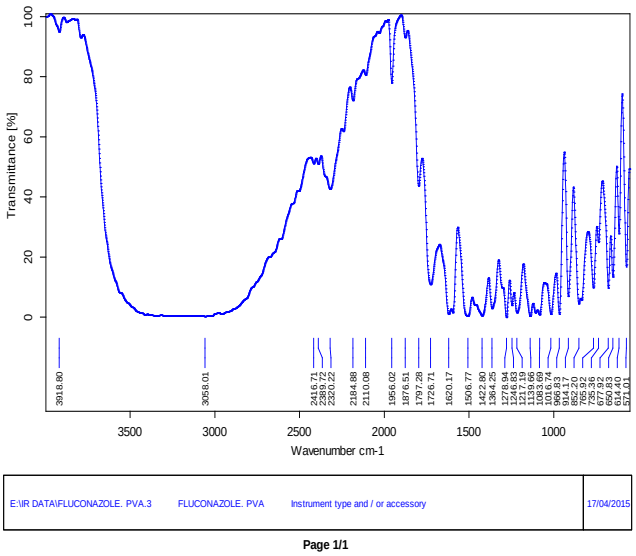

Fig. no: 12 FTIR for drug along with PVA

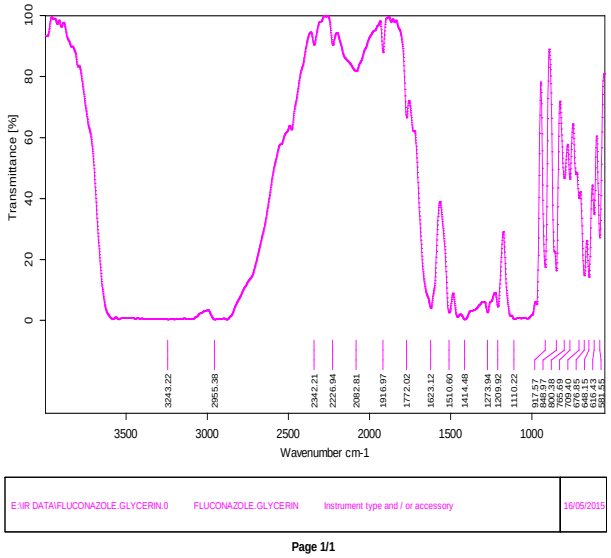

Fig. no: 13 FTIR for drug along with Glycerin

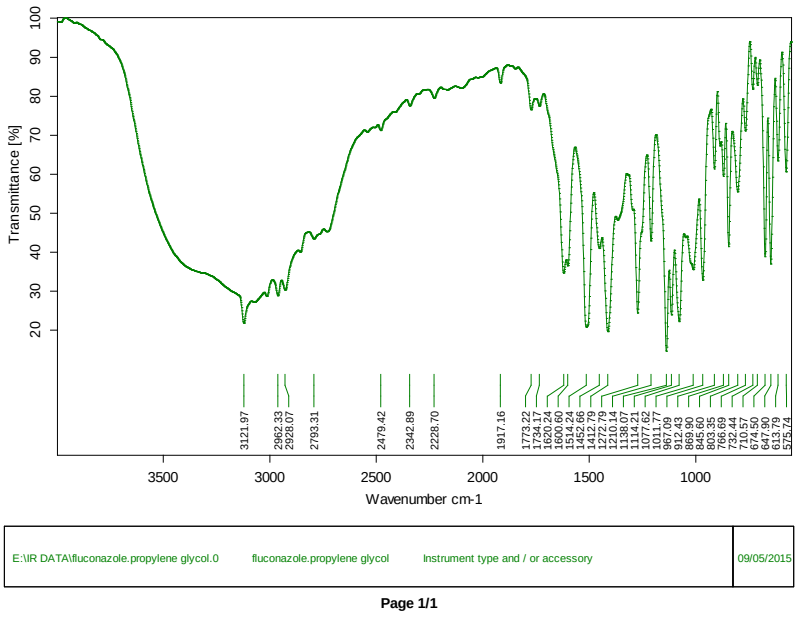

Fig. no: 14 FTIR for drug along with Propylene glycol

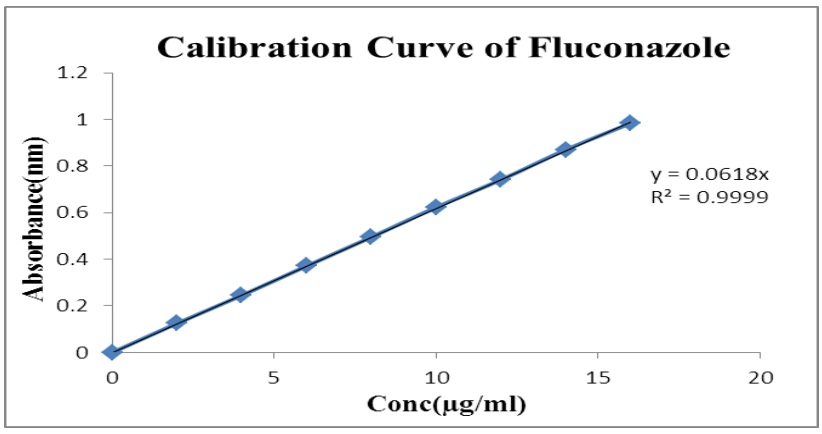

Figure No.15: Calibration Curve of Fluconazole

Table No.6: Release Kinetics Data of the Formulations F1-F11

Formulation code	Zero order R^2	First order R^2	Higuchi's R^2	Korsemeyer's	
				n	R^2
F1	0.947	0.951	0.931	0.675	0.976
F2	0.943	0.936	0.932	0.680	0.977
F3	0.994	0.928	0.687	0.650	0.972
F4	0.969	0.911	0.902	0.700	0.964
F5	0.994	0.921	0.881	0.829	0.986
F6	0.988	0.886	0.846	0.847	0.970
F7	0.987	0.929	0.846	0.638	0.966
F8	0.963	0.865	0.904	0.703	0.977
F9	0.964	0.934	0.915	0.696	0.976
F10	0.971	0.821	0.877	0.704	0.950
F11	0.955	0.856	0.919	0.673	0.964

The *In*-vitro release data was subjected to zero order, first order, Higuchis and Korsemeyers-Peppas models in order to establish the drug release mechanism and kinetics of the drug release from the Ocular inserts. When the data was subjected to zero order and first order kinetics model, a linear relationship was observed with high R^2 values for zero order model as compared to first order model and it suggested that the formulations followed zero order controlled release.

Higuchis model was applied to the *In*-vitro release data, linearity was obtained with high R^2 values suggested that the drug release from the Ocular inserts followed diffusion mechanism.

In order to define perfect model which will represent a better fit for *In*-vitro release data, Korsemeyers-Peppas model was applied which will define the exact mechanism. Good linearity with high R^2 values was observed with this model. The value of 'n' obtained for all the formulation was >0.5 and <1.0, suggesting that the drug release followed non-fickian diffusion.

CONCLUSION

In the present research, Fluconazole ocular inserts were used to treat the fungal infections of the eye were prepared by using Mercury substrate method. The prepared Fluconazole ocular inserts containing various concentrations of polymers and plasticizers were prepared by using Mercury Substrate Method. The prepared ocular inserts were evaluated for physicochemical parameters like uniformity of thickness, uniformity of weight, folding endurance, drug content, swelling index, % moisture absorption, % moisture loss, surface pH. Among all

prepared eleven formulations, F11 meets the required criteria and it's considered as the best formulation.

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