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FORMULATION AND EVALUATION OF ITRACONAZOLE OPTHALMIC *IN SITU* GELS

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Abstract:

Opthalmic In-situ gels refers to polymer solution which can be administered as liquid and undergoes a phase transition to semisolid gel upon exposure to physiological environment i.e., pH, Temperature, ions in the lachrymal fluid. The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug can be overcome by the use of in situ gel system that are instilled as drops into the eye and undergo a sol-gel transition in the cul-de-sac. Fungal keratitis is a serious disease that can lead to loss of vision if not diagnosed and treated effectively. Itraconazole is a triazole compound effective against Fungal keratitis. Because of poor bioavailability of Itraconazole opthalmic solutions, we formulated itraconazole opthalmic in situ gels by pH triggered, ion activation and temperature modulation methods. Total nine formulations have been prepared by using different concentrations of gelling agents i.e., carbopol 934 as pH triggered polymer, sodium alginate as ion activated polymer and poloxamer 407 as temperature triggered polymer. All the formulations contain Hydroxy Propyl Methyl Cellulose (HPMC) as viscosity enhancer, sodium chloride as tonicity adjusting agent, benzalkonium chloride as preservative. All the formulations were evaluated with respect to clarity, pH, gelling capacity, viscosity, drug content and in-vitro drug release. Among all the formulations, F4-F6 containing sodium alginate as gelling agent exhibited maximum drug release of 96.55±0.56% for prolonged period of time upto 8hrs. These formulations also shown optimum results for all the evaluation parameters. Hence formulations F4, F5 and F6 prepared by ion activation method were selected as optimized formulations. So from the present study, we concluded that the Itraconazole opthalmic in situ gels will be promising approach to overcome the drawbacks of conventional opthalmic solutions.

Keywords: *In Situ Gels, Carbopol 934, Poloxamer 407, Sodium Alginate, Itraconazole.*

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INTRODUCTION:

The ocular drug delivery system is considered as crucial and challenging because human eye is an isolated organ where the delivery of drug is more difficult. For the therapeutic treatment of most ocular problems, topical administration is the preferred route, because for systemically administered drugs, only a very small fraction of their total dose will reach the eye from the general circulatory system[1]. Even for this fraction, distribution to the inside of the eye is further hindered by the Blood-Retinal Barrier (BRB). A major problem being faced in ocular therapeutics is poor bioavailability of drugs and is mainly due to the frequent tear production, nonproductive absorption, poor residence time and impermeability of corneal epithelium[2]. Various ophthalmic vehicles such as Inserts, Ointments, Solutions, Suspensions and Aqueous gels, have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability. But these ocular drug delivery systems have not been used extensively because of various drawbacks such as poor drug bioavailability from solutions and suspensions, blurred vision from ointments or low patient compliance from ocular inserts[3,4].

Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. major problems associated with conventional ophthalmic formulations can be overcome by the use of *in situ* gel system which are instilled as drops into the eye and undergo a sol-gel transition in the cul-de-sac[5]. The sol-gel transition can be induced by a change in the physiological environment like shift in pH, temperature or ion activated systems. This type of gel combines the advantage of a solution (accurate and reproducible administration of drug) and gels (prolonged residence time) for enhancing ocular bioavailability[6].

In the present study we prepared opthalmic *in situ* gels of Itraconazole by three different methods of formulation. Itraconazole is a triazole compound effective against many fungal species including *Candida albicans* which is a causative organism of fungal keratitis[7]. Fungal keratitis is a serious disease that can lead to loss of vision if not diagnosed and treated effectively. The high penetration into the aqueous humor and low toxicity of Itraconazole make it a good candidate for consideration as a topical ocular antifungal agent.

MATERIALS AND METHODS:

Materials

Itraconazole was received as a gift sample from Hetero Pharma Ltd, Hyderabad. Poloxamer 407, Sodium alginate, Carbopol 934, Tween 60 and Hydroxy Propyl Methyl Cellulose K30 were procured from SD Fine Chem, Mumbai.

Methods

Drug - Polymer Compatibility Studies

Drug polymer compatibility studies were performed by using FTIR (Fourier Transform Infrared Spectroscopy). An infrared (IR) spectrum was obtained using the KBr disk method (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 $cm^{-1}$and the resolution was 1 cm^{-1}. FTIR absorption spectra of pure drug and all the polymers used like sodium alginate, carbopol 934, poloxamer 407 and HPMC K30 were obtained.

Formulation Development

Total nine formulations have been prepared by using different concentrations of gelling agents i.e., carbopol 934 as pH sensitive polymer, sodium alginate as ion activated polymer, poloxamer 407 as temperature triggered polymer. The composition of each and every formulation were shown in the formulation Table No.1. All the formulations contain Hydroxy Propyl Methyl Cellulose (HPMC) as viscosity enhancer, Sodium chloride as tonicity adjusting agent, TWEEN 60 as surfactant, benzalkonium chloride as preservative.

Table No.1 Formulation table of Itraconazole opthalmic *in situ* gels

Ingredients (gm)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Itraconazole	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HPMC K30	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Carbopol 934	0.2	0.3	0.4	-	-	-	-	-	-
Sodium Alginate	-	-	-	0.5	1.0	1.5	-	-	-
Polaxamers 407	-	-	-	-	-	-	15	20	25
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sodium Chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
TWEEN 60(ml)	1	1	1	1	1	1	1	1	1
Deionised Water (q.s in ml)	100	100	100	100	100	100	100	100	100

Different formulation methods used for preparing itraconazole opthalmic *in situ* gels are as follows:

pH triggered *in situ* gelation method: Formulations F1-F3 have been prepared by this method in which carbopol 934 has been used as pH triggered polymer. 0.9g of sodium chloride was dissolved in 50ml of water and HPMC K30 was added with continuous stirring by using magetic stirrer for one hour untill no lumps of HPMC should be observed. Then carbopol 934 was sprinlkled over the mixture and allowed for overnight. weighed quantity of drug was dissolved in TWEEN 60, Sodium chloride and benzalkonium chloride was added to this drug solution. Finally drug solution was added to the above polymer solution with continuous stirring[8].

Temperature triggered *in situ* gelation method: Formulations F4-F6 have been prepared by this method using poloxamer 407 as thermo triggered polymer. HPMC K30 was slowly added to 75 ml water with continous stirring, after stirring add the required amount of poloxamers and solution is stirred for 1 hr. Partially dissolved poloxamer solution were stored in refrigerator at 4^0C for 24 hrs. Finally drug solution as described in the earlier method was prepared and added to the above polymer solution[9].

Ion activated *in situ* gelation method: Sodium alginate has been used as ion triggered polymer in preparing formulations F7-F9. By using the magnetic stirrer different ratio of sodium alginate were dissolved in water and HPMC was added with continuous stirring until no lumps were appeared. Finally prepared drug solution was added to this polymer solution[10].

All the prepared nine formulations were filled in vials under aseptic conditions, sterilized in the autoclave (121° C and 15 psi) for 20 minutes and further evaluations were carried out[11].

Evaluation of Opthalmic *in situ* Gels: All the preparations were evaluated for various physicochemical properties.

- **Physical Appearance and clarity:** The physical appearance of the ophthalmic *in situ* gels has to be viewed by naked eye for characterizing the colour[12]. The clarity of formulated solution was determined by visual inspection under black & white background.
- **Determination of pH:** pH is one of the most important parameters involved in the ophthalmic formulation. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have a pH range between 6 to 7.4. The developed formulations were evaluated for pH by using Digital pH meter[13].
- ***In Vitro* Gelation Studies:** All prepared formulations were evaluated for gelling capacity in order to identify the compositions suitable for use as *in situ* gelling systems. The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of simulated tear fluid freshly prepared and equilibrated at 37^0C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve[14]. The composition of simulated tear fluid used was sodium chloride 0.670g, sodium bicarbonate 0.200g, calcium chloride 0.008g and purified water quantity sufficient to 100ml.
- **Rheological Studies:** Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye[15]. All the solutions were allowed to gel in Simulated Tear Fluid (STF) and then viscosity was measured. Viscosity of solution and gels was measured by Brook field viscometer type DV-II + PRO using spindle no.61 and 63.
- **Drug Content:** The drug content of *in situ* gel was determined by taking sample (5 ml) of *in situ* gel and diluted with STF. Then the absorbance was measured at 261.6 nm using UV visible spectrophotometer to calculate the percentage of drug content[16].
- **Sterility Test:** Sterility testing was performed for the aerobic, anaerobic bacteria and fungi by using fluid thioglycolate medium and soybean-casein digest medium respectively as per the Indian Pharmacopoeia 2014. The 1ml sterile optimized formulation was taken and diluted with 100ml sterile water for injection[17]. From this, 5ml solution was added in each medium and incubated for not less than 14 days at 20-25^0C in the fluid thioglycolate medium and at 20-25^0C in soyabean casein digest medium to find out growth of any microorganisms in the formulation.
- ***In Vitro* drug Release Studies:** The Franz-diffusion cell was used for studying *in vitro* drug release of *in situ* gels. Egg membrane were used as biological membrane for diffusion studies. Egg membrane was attached to the terminal portion of the cylindrical donor compartment. 1ml *in situ* gel containing drug, sufficient for establishing sink conditions of the assay was placed into the donor compartment. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The receptor compartment was filled with Simulated Tear Fluid (STF) of pH 7.4 as diffusion medium and maintained at 37^0C under mild agitation using magnetic stirrer. At specific time intervals, aliquots of 1 ml were withdrawn and immediately restored with the same volume of fresh STF. The amount of drug released was measured by using UV Visible spectrophotometer[18].

RESULTS AND DISCUSSION:

Drug - Polymer Compatibility Studies: In the physical mixture of Itraconazole with sodium alginate, carbopol 934 and Poloxamer 407 the major peaks belonging to drug functional groups were obtained almost at the same wave numbers. However, additional peaks were obtained in physical mixtures which could be due to presence of impurities but there is no influence in the drug peaks, which indicates that there is lack of significant interaction between the drug and polymers and the entrapment of drug is only by physical process.

Evaluation of *In-Situ* Ophthalmic Gels: All the *in situ* gel formulations were evaluated for physical appearance, clarity, pH, drug content, gelling capacity, rheological study, sterility testing and *in vitro* diffusion study.

Physical appearance and clarity: All Formulations were checked for its clarity under dark background and also for visual appearance. Formulations F1-F6 were found to be clear and transparent where as Formulations F7-F9 were found to be creamy white solutions may be due to high concentration of poloxamer 407.

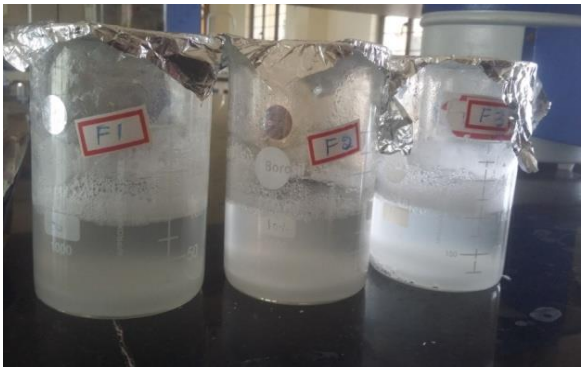

Fig 1: Physical Appearance of Formulations F1-F3

Determination of pH: pH of all the formulations were found between 6.04 to 7.24. As the results are within the acceptable range, there won't be any irritation to the patient after administration of formulation. pH values of all the formulations were shown in the Table No.2.

Determination of drug content: Drug content of all the formulations were found to be in the acceptable range. The drug content values are ranging from 92.13±0.17% to 98.88±0.08% and the results were shown in the table 2.

Table No. 2 pH and drug content of formulations F1-F9

Formulation code	pH(mean ± S.D)	Drug Content (% w/w) (mean ± S.D)
F1	6.08±0.1	97.19±0.88
F2	6.04±0.25	96.38±0.56
F3	6.11±0.21	95.1±0.21
F4	7.12±0.05	94.20±0.98
F5	7.24±0.07	98.88±0.08
F6	7.31±0.23	98.16±0.76
F7	6.93±0.08	95.33±0.65
F8	7.14±0.06	94.61±0.33
F9	7.02±0.03	92.13±0.17

Table No.3 *In vitro* Gelling Capacity of formulations F1-F9

S.No.	Formulation Code	Gelling Capacity
1	F1	+
2	F2	++
3	F3	++
4	F4	++
5	F5	+++
6	F6	+++
7	F7	+++
8	F8	+++
9	F9	+++

Evaluation of gelling capacity: All the prepared formulations were evaluated for its gelling capacity in stimulated tear fluid having pH 7.4 and results were shown in the Table No.3. Formulations containing carbopol 934 as gelling agent (F1-F3) forming gels within minutes but show rapid dissolution. Formulations containing sodium alginate as gelling agent (F4-F6) and poloxamer 407 as gelling agent (F7-F9) were showing very good gelling capacity which remains its gelling strength for extended period as shown in Fig. No.2.

- o gelation : -
- elation occured in few minutes and dispersed rapidly :
- elation immediate within seconds, remained for few hours <6-7 hrs : ++
- elation immediate, instantly and for extended period more than 10 hrs : +++

Fig. No. 2 Transparent gel formation of formulations F4-F6

Rheological Studies: Viscosity of the formulations governs the behavior of the formulations in the cul-de-sac resisting against the hydrodynamism and the blinking of the eye. In order to evaluate the rheological behavior, viscosity of the formulation before and after addition of simulated lachrimal fluid was evaluated using Brookfield viscometer. All the formulations were found to be shear thinning exhibiting pseudoplastic behavior. All the formulations were liquid at room temperature and underwent rapid gelation upon raising the pH to 7.4 . The samples were analyzed both at room temperature at 25°C and also at 37 ± 0.5°C. Results of viscosity studies before and after gelation were presented in the Table No.4.

For ion activated *in-situ* gelling formulations (F4-F6), the viscosity values at 37°C after dilution with simulated tears was much higher than those at 25°C without simulated tears suggesting the occurrence of phase transition. Alginate contains moieties of α-L-guluronic acid (G) which interact with the calcium N ions in tear fluid to form three dimensional ionotropic hydrogel matrices. Hence, liquid formulations G containing alginate undergo rapid transition into the gel phase with high viscosity on exposure to divalent + cations in tear fluid.

G The viscosity of temperature triggered *in-situ* gelling formulations (F7-F9) increased with increasing the temperature from the non-physiological temperature G (25°C) to the physiological one (37°C), and the conversion of solution to semisolid gel was observed confirming *in-situ* gelling characteristics. This reversible sol-gel transition behavior at high temperature may be due to aggregation between the polyethylene oxide (PEO) and polypropylene oxide (PPO) moieties of Poloxamer 407 to form micelles. When the concentration and the temperature of Poloxamer 407 aqueous solutions are above the critical values, the molecules will arrange to form micelles with a dehydrated PPO core surrounded by hydrated swollen PEO chains imparting an increase in the viscosity.

Viscosity of pH triggered formulations (F1-F3) was increased due to pH change after instillation of the formulation into the tear film leads to an almost instantaneous transformation of the highly fluid latex into a viscous gel. Swelling of hydrogel increases as the external pH increases.

From the results we observed that all the nine formulations exhibited thixotropic behavior but the change in viscosity was very less in formualtions (F1-F3), very high in formualtions (F4-F6) and optimum viscosity change was observed in formualtions (F7-F9).

Table No.4 Viscosity of all Formulations Before and after Gelation

Formulation code	Viscosity of solution at 25°C (in cps) (mean ± S.D)	Viscosity of *in situ* gel at 37°C (in cps) (mean ± S.D)
F1	172.8± 1.51	296±3.61
F2	259±2.64	409±4.22
F3	355± 3.72	493±2.34
F4	1435.7±5.69	2633.33±3.23
F5	1321.6±5.64	4380.53±3.57
F6	950±4.87	2553.16±2.51
F7	98±2.98	41690.23±3.18
F8	58±4.63	11554.22±2.63
F9	132±2.02	18350.18±2.38

Sterility testing:
All the prepared formulations were evaluated for its sterility by using fluid thioglycolate medium and soybean-casein digest medium respectively. After the incubation period, all the formulations were checked for the growth of any microorganism in the respective mediums. All the prepared formulations were found visually there is no growth of any bacteria or fungi. Hence all the formulations are sterile and found to be free from microorganisms.

***In Vitro* drug Release Studies:** The *in vitro* diffusion of itraconazole from the prepared formulation was studied using Franz diffusion cell using STF of pH 7.4 as diffusion medium. Egg membrane placed between receptor and donor compartment was used as diffusion membrane. The diffusion studies of prepared *in situ* gelling systems were carried up to 8 hours. Withdrawn samples at regular intervals have been evaluated using UV Visible spectrophotometer. Results of drug release of all formulations were shown in the Table No.5 and 6. Comparative *in vitro* drug release plots of all formulations were shown in Figure No.3 and 4.

Table No. 5 *In Vitro* Drug Release Data of Formulations F1-F6

Time (in mins)	F1	F2	F3	F4	F5	F6
15	9.21±0.13	7.63±0.55	6.54±0.32	7.65±0.15	4.47±0.35	8.86±0.72
30	15.72±0.34	13.25±0.64	11.74±0.21	15.82±0.11	11.00±0.16	15.75±0.09
45	23.87±0.12	22.90±0.23	16.71±0.24	22.23±0.21	25.13±0.12	23.97±0.25
60	39.27±0.63	36.71±0.19	38.17±0.09	38.73±0.27	36.89±0.24	28.03±0.33
120	56.43±0.23	42.69±0.34	46.06±0.32	49.88±0.65	53.84±0.15	34.80±0.11
180	70.80±0.31	68.51±0.78	66.51±0.23	56.98±0.34	61.35±0.27	43.85±0.15
240	84.32±0.35	80.28±0.82	76.45±0.18	65.37±0.18	70.79±0.34	56.67±0.18
360	90.51±0.56	91.08±0.77	85.48±0.43	75.57±0.25	79.72±0.42	69.00±0.29
420	-	-	92.88±0.50	82.99±0.02	87.90±0.15	83.13±0.23
480	-	-	-	93.16±0.32	96.55±0.56	94.64±0.15

Table No.6 *In Vitro* Drug Release Data of Formulations F7-F9

Time (in mins)	F7	F8	F9
15	5.79±0.24	4.10±0.47	3.45±0.20
30	9.62±0.33	9.33±0.24	10.81±0.32
45	18.91±0.87	12.48±0.51	23.49±0.29
60	27.62±0.12	27.29±0.22	30.95±0.57
120	38.78±0.34	36.65±0.27	39.66±0.33
180	53.66±0.17	45.81±0.32	45.66±0.33
240	62.32±0.12	50.30±0.35	49.27±0.76
360	77.78±0.34	58.74±0.14	53.40±0.11
420	82.91±0.45	67.63±0.12	58.06±0.63
480	87.19±0.62	78.65±0.20	62.56±0.42

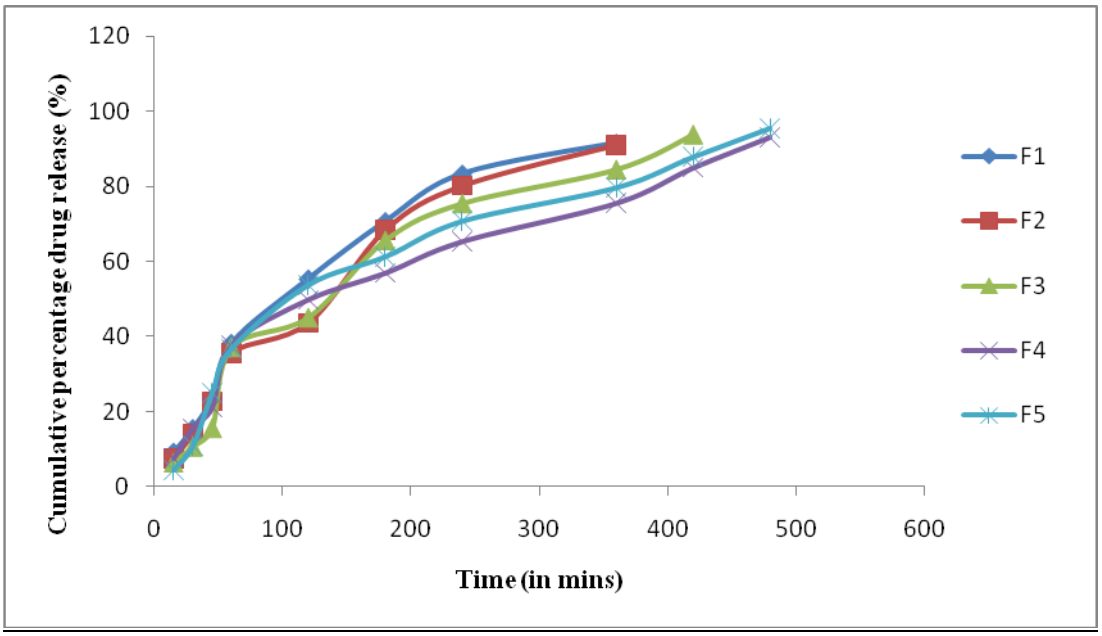

Figure No.3 Comparative *In Vitro* Drug Release of Formulations F1-F5

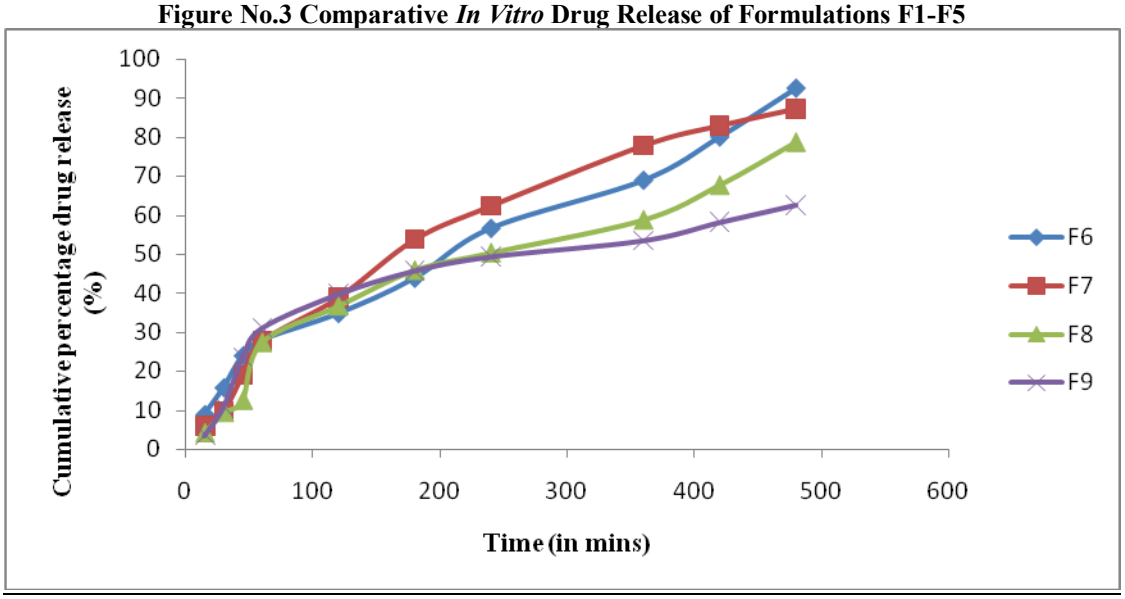

Figure No.4 Comparative *In Vitro* Drug Release of Formulations F6-F9

Formulations containing carbopol 934 shows drug release upto 7hrs with maximum of 92.78±0.50% of cumulative percentage drug release at the end of 7th hour. Rapid drug release of these formulations were mainly due to the rapid dissolution of formed *in situ* gels. All the formulations containing sodium alginate exhibit drug release upto 8hrs with maximum of 96.55±0.56% of cumulative percentage drug release at the end of 8th hour. Because of its optimum viscosity and good gelling capacity drug release was prolonged for extended period of time. Formulations containing poloxamer 407 shows drug release for 8hrs but with maximum of 87.19±0.62% of cumulative percentage drug release at the end of 8th hour. This is may be due to the high viscosity of formulations after gelation which retards the drug release from the formed gels. So the formulations containing sodium alginate as gelling agent (F4-F6) exhibited maximum drug release for prolonged period of time. These formulations also show optimum results for all the evaluation parameters. Hence formulations F4, F5 and F6 were selected as optimized formulations.

CONCLUSION:

From this study we concluded that Itraconazole opthalmic *in situ* gels improves drug bioavailability by increasing drug retention time with prolonged drug release in the eye. Opthalmic *in situ* gels will be promising approach to overcome the drawbacks of conventional opthalmic solutions. Hence further work is recommended to support its efficacy claims by *in vivo* studies and stability studies.

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