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Original Research Article

Design and Development of Ofloxacin Insitu Gel Using Mucoadhesive Polymers

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Abstract: In ocular delivery the physiological constraints imposed by the protective mechanisms of the eye lead to low absorption of drugs, resulting in a short duration of the therapeutic effect, poor bioavailability exhibited by these conventional eye drops. Thus the rapid pre corneal elimination of the drug can be overcome by these in situ gelling systems that are instilled as drops into the eye and undergo a sol-to-gel transition in the cul-de-sac and improves the residence time of the drug in the eye is increased, bioavailability and patient compliance compare to eye drops. The purpose of the present research work was the optimization and evaluation of pH induced ophthalmic in-situ gel of Ofloxacin. The in-situ gel formulation were prepared by simple mixing method based on the pH triggering system by incorporation of various polymers like HPMC K10, HPMC K100 and Carbopol-940 in different proportions. The *in-situ* gel characteristics were evaluated for Clarity, gel pH, gel Capacity, Viscosity, sterility testing and in-vitro drug release studies. Optimization was done by using in-vitro diffusion study. The optimized formula showed no significant changes on stability studies when stored at 40ºC/75% RH for one month according to ICH guidelines.
Keywords: Ophthalmic *in-situ* gel, Ofloxacin, Carbopol-940, HPMC K10, HPMC K100.

INTRODUCTION

Eye is one of the challenging organs for drug delivery because of its unique anatomy restricts drug absorption into deeper tissues. Poor bioavailability of drugs from conventional ocular dosage forms is mainly due to tear production, non-productive absorption, transient residence time, and impermeability of corneal epithelium, binding by the lachrymal proteins, drainage of the instilled solution, tear turnover and limited corneal area [1]. Several Novel drug delivery systems have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface but also to slow down drug elimination. Successful results have been obtained with inserts and collagen shields However; these preparations have some disadvantages such as poor compliance, especially by elderly people and many patients sometimes lose the device without noticing it. Nowadays, a major progress in development of ophthalmic formulations has been performed by the ophthalmic gel technology i.e in the development of "in situ gel" which consists of certain polymers undergoing sol–gel phase transition in response to environmental conditions such as pH, specific ions and temperature.

In particular [2], a thermo responsive in situ gel, an ophthalmic product vehicle responding to a shift in temperature, possesses liquid characteristic at room temperature and becomes gel when comes in contact with body temperature. One of well-known polymer types possessing thermo responsive behavior is Pluronics, so called Poloxamers. They are a triblock copolymer poly (ethylene oxide)-bpoly (propylene oxide) - b-poly (ethylene oxide) (PEO–PPO–PEO) showing amphiphilic behavior due to hydrophilic ethylene oxide domains and hydrophobic propylene oxide domains. The gelation mechanism of Pluronics could be explained by the changes in micellar structure as a function of concentration and temperature.3-5 However, a major disadvantage of Pluronics is their low mucoadhesive activity, therefore, some Pluronic-based ophthalmic formulations have been improved by adding polymers providing mucoadhesive property such as cellulose derivatives.4 Levofloxacin hemihydrates is a fluoroquinolone derivative used to treat external infections of eye such as acute and subacute bacterial conjunctivitis, keratitis, keratoconjuctivitis and corneal ulcers.

MATERIALS AND METHODS

Ofloxacin was obtained as gift samples from MSN labs, Hyderabad. All other chemicals were purchased from Chemspure, Chennai which were of analytical grade.

Table-1: Composition of Ophthalmic *Insitu* Gels

Ingredients	F1	F2	F3	F4	F5	F6
Ofloxacin (gm)	300	300	300	300	300	300
Carbapol -940 (gm)	0.5	0.5 gm	0.5	0.5	0.5	0.5
HPMC $_{K10}$ (gm)	0.3	0.4	---	0.2	0.1	0.5
HPMC $_{K100}$ (gm)	0.2	0.1 gm	0.5	0.3	0.4	---
Citric Acid (gm)	0.407	0.407	0.407	0.407	0.407	0.407
Di sodium hydrogen phosphate (gm)	1.125	1.125	1.125	1.125	1.125	1.125
Sodium Hydroxide (gm)	0.1	0.1	0.1	0.1	0.1	0.1
Tween 80 (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Benzalkonium Chloride	0.002	0.002	0.002	0.002	0.002	0.002
Distilled Water (ml)	100	100	100	100	100	100

EXPERIMENTAL WORK

Preparation of *insitu* gels:

Different concentrations of polymers [3] were used to prepare ophthalmic solutions as per the composition shown in Table no 1. The polymers were dissolved in citrophosphate buffer and allowed to hydrate. Ofloxacin was dissolved in sodium hydroxide solution (0.1N) and was dissolved in acetone separately. The drug solutions were then added to the polymeric solution under constant stirring until a uniform solution was obtained. Benzalkonium chloride was added as preservative. Distilled water was then added to make up the final volume. The formulations were filled in vials under aseptic conditions, sterilized in the autoclave (121º C and 15 psi) for 20 minutes and further evaluations were carried out [4].

Evaluation of *insitu* gels:

The *insitu* gels were evaluated for Clarity, Appearance, pH, Gelling capacity, Drug content, Rheological studies, *in vitro* release studies and Stability.

Clarity:

All developed formulations were evaluated for clarity by visual observation against a black and white background.

pH:

Ophthalmic formulations should have pH range in between 5 to 7.4. The developed formulations were evaluated for pH by using digital pH meter**.**

Gelling Capacity [5]:

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37ºC and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

Drug Content:

The drug content was determined by diluting 1 ml of the formulation to 100 ml with STF solution p^H 7.4. Aliquot of 1 ml was withdrawn and further diluted to 10 ml with STF. Ofloxacin concentration was then determined by simultaneous method at 294 nm by using UV-Vis spectrophotometer.

Rheological Studies [7]:

The formulations were poured into the sample adaptor of the Brookfield DV Erheomoter and angular velocity was increased gradually from 1 to 50 rpm using spindle no. 4. The hierarchy of angular velocity was reversed and the average dial reading was considered to calculate the viscosity. The temperature was maintained within 37 ± 0.1°C.

In vitro release (diffusion) studies:

The *in vitro* release of Ofloxacin and from the formulations was studied through biological egg membrane using a fabricated dissolution testing apparatus [8]. The dissolution medium used was artificial tear fluid freshly prepared (p^H 7.4). Biological egg membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter). A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was suspended in 50 ml of dissolution medium maintained at 37ºC so that the membrane just touched the receptor medium surface. The dissolution

medium was stirred at 50 rpm using magnetic stirrer. Aliquots, each of 1 ml volume, were withdrawn at regular intervals and replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 294 nm with the use of simultaneous estimation method [9].

In order to find out the order of release and the mechanism, which predominantly influences the drug release, the in-vitro drug release data was subjected to the different models of graphical treatment.

KINETICS OF DRUG RELEASE [10]

To study the study kinetics, data obtained from in vitro release were plotted in various kinetic models.

Zero order equation

The graph was plotted as % drug released Vs time in hours.

$$C=K_0t$$

Where,

K_0 – Zero order constant in concentration/time

t – Time in hours

The graph would yield a straight line with a slope equal to K_0 and intercept the origin of the axis.

First order equation

The graph was plotted as log % cumulative drug remaining Vs Time in hours.

$$\text{Log } C = \log C_0 - Kt/2.303$$

Where,

C_0 - initial concentration of drug

K- First order constant.

t- Time.

Higuchi kinetics [11]

The graph was plotted as % Cumulative drug released Vs square root of time

$$Q = Kt^{1/2}$$

Where,

K – Constant reflecting design variable system.

t - Time in hours.

Hixson and crowell erosion equation

To evaluate the drug release with changes in the surface area and the diameter of particles, the data were plotted using the Hixson and crowell rate equation. The graph was plotted by cube root of % drug remaining Vs time in hours.

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC} X t$$

Where,

Q_t – Amount of drug released in time t.

Q_0 - Initial amount of drug

K_{HC} – Rate constant for Hixon crowell equation

Korsmeyer – Peppas equation [12]

To evaluate the mechanism of drug release, it was further plotted in peppas equation as log cumulative % of drug released Vs time

$$M_t / M_\alpha = Kt^n$$

$$\text{Log } M_t / M_\alpha = \log K + n \log t$$

Where,

M_t / M_α - fraction of drug released at time t

t – Release time

K – Kinetic constant (incorporating structural and geometric characteristics of preparation)

n - Diffusional exponent indicative of the mechanism drug release.

Table 3: Mechanism of drug profile

n	Mechanism
0.5	Fickian diffusion (Higuchi matrix) Matrix)
0.5 < n < 1	Non-Fickian diffusion
1	Case II transport

The n value could be obtained from slope of the plot of log cumulative % of drug released Vs log time. The results are given in Table no-.

STABILITY STUDIES [13, 14]

The accelerated stability studies were carried out according to the ICH guidelines. Optimized formulation F6 was sealed in amber colored bottles with cap covered by aluminum foil and these packed formulation was stored in ICH certified stability chambers (Thermo labs, Mumbai) maintained at 40ºC ± 2ºC and 75% RH ± 5 % for a month. The formulations were evaluated before and after periodic interval for change in appearance, drug content, and *In vitro* drug release. Table no 13 & 14

RESULTS AND DISCUSSION

PREFORMULATION STUDIES

Melting point:

Melting point of both the drugs complies with the standards, thus indicating the purity of the drug samples (Table no-4).

Loss on drying:

Loss on drying of both the drugs complies with the standards, thus indicating the purity of the drug samples (Table no- 4).

Table 4: Melting point and Loss on drying of Ofloxacin and Dexamethasone

S.NO	TEST	DRUGS
		OFLOXACIN
1.	Melting point	263ºC
2.	Loss on drying	0.15%

Analysis of Ofloxacin:

The IR spectrum of Ofloxacin shown in Fig 10, reveals characteristic peaks in the Ofloxacin IR spectrum that occur at 696.12 cm^{-1} for the Aromatic -C-H Bending ,1719.90 cm^{-1} for the C=O stretching, 1303.41 $cm^{-1}$for the C-F stretching, 2777.57 cm^{-1} for the O-H stretching respectively .Fig no-1

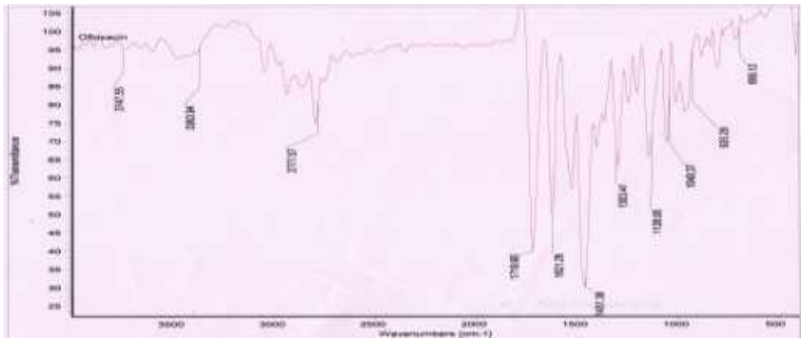

Fig 1: FT-IR spectrum of Ofloxacin

Fig 2: FT-IR spectrum of HPMC

Study of interaction of the drugs with excipients:

The peaks obtained in physical mixture spectrum matches with the peaks obtained in the spectrum of Ofloxacin, as shown in Fig no3& 4. Therefore it can be concluded that there is no interaction of drugs with excipients.

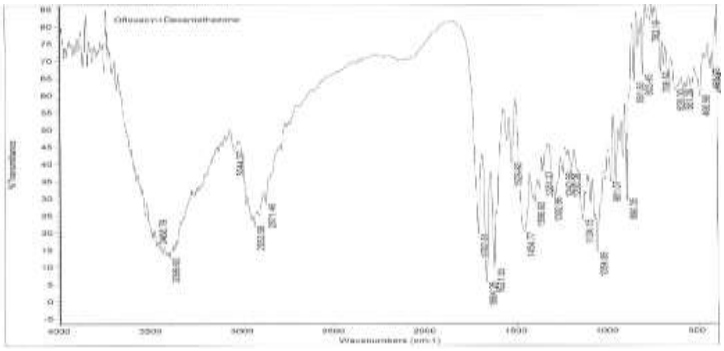

Fig 3: FT-IR spectrum of Ofloxacin and Dexamethasone

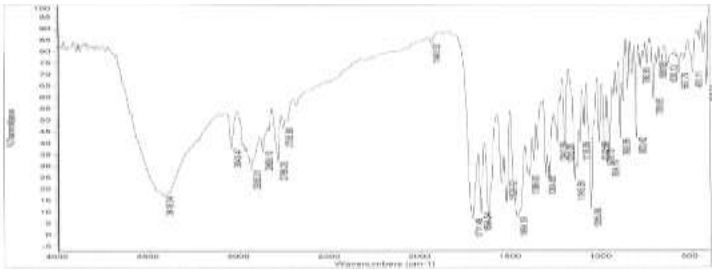

Fig 4: FT-IR spectrum of Physical mixture

STANDARD CALIBRATION CURVE OF OFLOXACIN:

It was found that the estimation of Ofloxacin by UV Spectrophotometric method at λ_{max}294.0 nm STF p^H 7.4 had good reproducibility and this method was used in the study. The correlation coefficient for the standard curve was found to be closer to 1 that is 0.997, at the concentration range, 1-5 µg/ml. The regression equation generated was y = 0.0806x (table no-5 & Fig no-5)

Table 5: Standard curve of Ofloxacin in simulated tear fluid p^H 7.4

S.No	Concentration (mcg/ml)	Absorbance at 294nm
1.	1	0.089
2.	2	0.167
3.	3	0.245
4.	4	0.329
5.	5	0.392

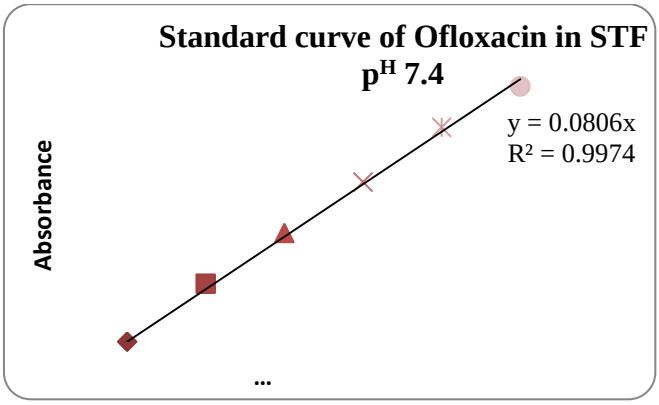

Fig 5: Standard curve of Ofloxacin simulated tear fluid p^H 7.4

Formulation of ophthalmic in-situ gels:

Insitu gels were formulated by using simple mixing method it's a cooling technique. In this method, Carbopol-940 was used as a controlled delivery component, HPMC was used as a viscosity enhancer and BKC used as preservative, citric acid and disodium hydrogen phosphate used as buffer salts (Table no-1).

Evaluation Parameters:

Clarity and Appearance:

The physical appearance of all the developed formulations are light yellow and are clear (Table no-7). All the developed gels show good homogeneity with absence of lumps. Terminal sterilization by autoclaving had left no effect on appearance and other physicochemical properties of all formulations.

p^H:

The p^H of all the formulations found to be in the range of 5.99 to 6.42 (Table no- 7).Terminal sterilization by autoclaving had left no effect on p^H.

Gelling Capacity:

The time for gelation and the time taken for the gel formed to dissolve is noted as given in table no-7.

Table 7: Values of evaluation parameters of Formulation F1-F6

Formulation code	Gelling capacity at 25^0C	Gelling capacity at 37^0C
F1	-	++
F2	-	+
F3	-	+++
F4	-	+++
F5	-	++
F6	-	+

-: No gelation
+: gels after few min and dissolves rapidly
++: gelation immediate, remains for few hrs.
+++: gelation immediate, remains for extends period

Drug content:

The drug content was determined for all formulations and results were shown in table no-8. The percent drug content was found to be in between 88.33% to 98.66% for Ofloxacin.

Table 8: Drug content of formulations F1-F9

S.no	Formulation code	% Drug content
		Ofloxacin
1	F1	88.33
2	F2	89.01
3	F3	90.66
4	F4	90.33
5	F5	91.66
6	F6	93.33
7	F7	98.66
8	F8	93.66
9	F9	94.05

Rheological Studies:

Viscosity was determined for all formulations at different RPM and results were shown in table no- 9 & fig no-7.The gels are typically pseudo plastic, exhibiting non-Newtonian flow (shear thining) characterized by decreasing viscosity with increasing the shear rate.

Table 9: Rheological study of formulations F1-F6

S.no	Formulation code	Viscosity at different RPM(cps)		
		10	25	50
1	F1	110	92	75
2	F2	141	115	95
3	F3	161	135	120
4	F4	139	121	99
5	F5	153	149	118
6	F6	185	158	137

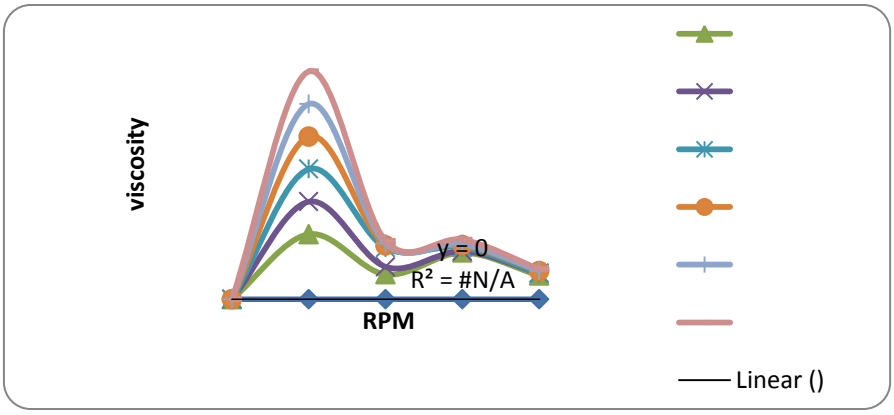

Fig-6: Viscosity of formulations F1- F6 at different RPM

In-vitro Drug Release Studies from in-situ gels:

The Carbopol resins are hydrophilic substances that are not soluble in water. Rather, these polymers swell when dispersed in water forming a colloidal, mucilage-like dispersion. Carbopol readily absorb water, get hydrated and swell. In addition to its hydrophilic nature, its cross-linked structure and although they are described as soluble carbopol do not dissolve but merely swell to a remarkable extent this makes Carbopol a potential candidate for use in controlled release drug delivery system. In vitro diffusion study for all formulations was carried out using fabricated dissolution testing apparatus. The percentage cumulative release of ofloxacin from formulations F1-F9 was found to be in between 62.56% to 95.55% .The results were shown in table no-10,11 & fig no- 8 to 11.

COMPARITIVE IN VITRO DIFFUSION STUDY OF OFLIOXACIN F1-F6 FORMULATIONS:

Overall curve fitting showed that the Ofloxacin release from gels followed Korsmeyer-peppas model except the formulations F3 and F9 which followed Higuchi model. The critical value of n ranging between 0.6473-0.8274 suggests non-Fickian diffusion (Table no-12).

STABILITY STUDY:

***In vitro* release of Ofloxacin from formulation F1-F6 in STF p^H 7.4 Table no-10**

Time	F1	F2	F3	F4	F5	F6
30	9.31±0.13	7.53±0.55	6.44±0.32	8.86±0.72	7.55±0.15	5.79±0.24
60	15.62±0.34	14.25±0.64	10.63±0.21	15.75±0.09	11.48±0.18	9.62±0.33
90	23.77±0.12	22.80±0.23	15.61±0.24	23.97±0.25	15.72±0.11	14.45±0.76
120	29.13±0.45	27.87±0.12	24.75±0.56	28.03±0.33	21.22±0.21	18.91±0.87
150	38.27±0.63	35.71±0.19	37.17±0.09	34.80±0.11	32.73±0.27	27.62±0.12
180	55.43±0.23	43.69±0.34	45.05±0.32	43.85±0.15	37.14±0.18	35.81±0.24
210	70.70±0.31	54.25±0.21	56.42±0.14	56.67±0.18	49.89±0.65	48.78±0.34
240	83.32±0.35	68.41±0.78	65.61±0.23	69.00±0.29	56.98±0.34	53.66±0.17
270	91.51±0.56	79.18±0.82	75.44±0.18	80.13±0.23	65.37±0.18	62.32±0.12
300		93.06±0.77	84.49±0.43	92.64±0.15	75.57±0.25	70.44±0.23
330			91.78±0.50		84.99±0.02	77.78±0.34
360					93.16±0.32	82.91±0.45

The stability studies were executed for optimized formulation. Studies were done for one month and observed for appearance, p^H, drug content, gelling capacity and invitro drug release. The appearance was clear and no significant variation in p^H was observed. Gelling capacity, drug content and drug release also shows no significant variations. Given in Table no-10, fig no-8

Comparitive in vitro diffusion study of Ofloxacin F1-F6 Formulations fig no-8

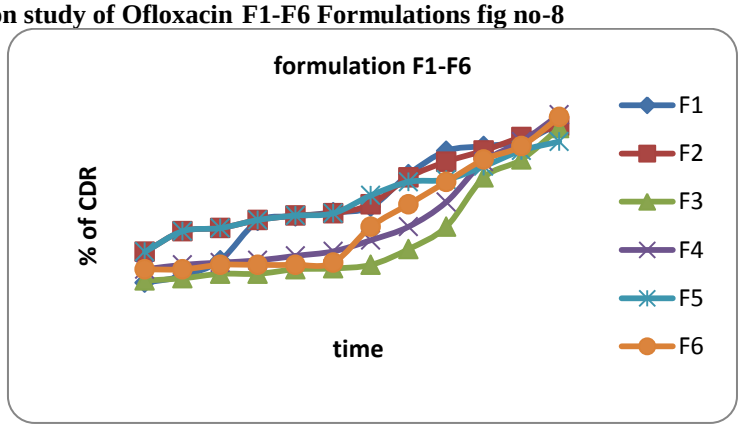

Fig-8

Drug release kinetics of Formulation F6 table no-11

OFLOXACIN & DEXAMETHASONE							
Formulation code	Zero order R^2	First order R^2	Higuchi R^2	Hixson-crowell R^2	Korsmeyer peppas		Best fit model
					n	R^2	
F4	0.9876	0.8890	0.9838	0.9247	0.8274	0.9989	Korsmeyer peppas

STATISTICAL ANALYSIS:

Overall curve fitting showed that the Ofloxacin release from gels followed Korsmeyer-peppas model except the formulations F7 which followed Higuchi model. The critical value of n ranging 0.8274 suggests non-Fickian diffusion. Table no no-11 & Fig no-9, 10

Zero order kinetics of optimized formulaF7: Korsmeyer-Peppas plot of optimized formulaF7

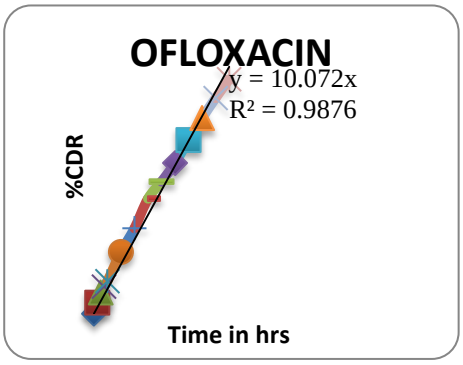

Fig 9

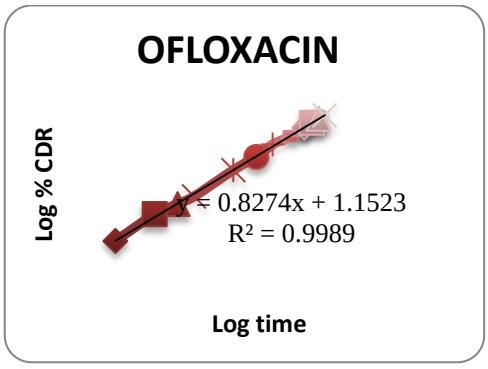

Fig 10

Microbial efficacy studies:

The insitu gelling formulations showed antimicrobial activity when tested microbiologically by disc technique. Clear zones of inhibition were obtained.

The diameter of zone of inhibition produced by formulations against Staphylococcus aureus is given in Table -12.

Fig 18: Antimicrobial activity of F1-F6 Formulations

Table 12: anti microbial activity of F1-F6 Formuations

Test organism	Diameter of Zone of Inhibition(mm)						
	Std	F1	F2	F3	F4	F5	F6
Staphylococcus aureus	23	6	15	7	19	8	9

STABILITY STUDY:

Table 13 Physicochemical properties of optimized formulation F6

Time	Appearance	Clarity	Gel p^H	Drug content
				Ofloxacin
Initial	Light yellow	Clear	6.01	98.66
After 1st Month	Light yellow	Clear	6.00	98.28

Table 14: In vitro drug diffusion studies of optimized formulation F6

Time (min)	Cum % Drug release	
	Initial	After 1 month
	Ofloxacin	Ofloxacin
30	4.47±0.35	4.03±0.23
60	8.59±0.22	8.36±0.42
90	11.00±0.16	10.54±0.32
120	13.23±0.31	13.21±0.25
180	25.13±0.12	24.66±0.12
240	34.80±0.06	33.87±0.23
300	46.89±0.24	46.62±0.45
360	53.84±0.15	53.78±0.37

SUMMARY & CONCLUSION

In the present work, an attempt has been made to develop ophthalmic in-situ gel of Ofloxacin to optimize using invitro diffusion study. The IR spectra revealed that, there was no interaction between drugs and polymers. All polymers used were compatible with drug. Simple mixing method that is cooling technique was employed to formulate the opthalmic in-situ gels. The evaluation parameters of the in-situ gel like gel p^H, gel capacity, clarity, drug content, viscosity and in-vitro drug release studies were carried out. All the parameters were found to be within the limits. From the data obtained, it is observed that amongst the various combinations of the polymers used in the study, in-situ gels that were formulated using Carbopol-940 (0.6gms) and HPMCK10 (0.5gm), HPMCK100 exhibited better results than compared to those other combination of polymers in different concentration. The effectiveness of polymers (Carbopol-940 and HPMCK100) on the drug release was explained. Antimicrobial efficacy study was carried out using Staphylococcus aureus as test microorganism.

After incubation up to 24 hours, it was found that F4 had effective antimicrobial action as that of standard. The observed independent variables were found to be very close to predicted values of optimized formulation which demonstrates the feasibility of the optimization procedure in successful development of opthalmic in-situ gel containing Ofloxacin by using Carbopol-940 and $HPMCK_{100}$. The drug release form the optimized formula was found to be diffusion controlled and n value of Peppas equation for Ofloxacin was0.8274, which indicates non-fickian diffusion controlled mechanism. The stability studies revealed that there was no significant change in in-situ gel properties with aging at different storage conditions. It is concluded from the present studies that the in-situ gels of Ofloxacin was capable of exhibiting controlled release with stability and the optimized formulation Carbopol-940 and HPMC (0.6g and 0.5g) has fulfilled the objectives of the present study like reduction in the frequency of administration, improved patient compliance.

REFERENCES

1. M. N. V. Ravi Kumar, Handbook of Particulate Drug Delivery, American Scientific Publishers.2008: 2.300-305.
2. Wang NX, Von Recum HA. Affinity-Based Drug Delivery. Macromolecular bioscience. 2011 Mar 10; 11(3):321-32.
3. Robinson J.R In; Ophthalmic drug delivery system.1933.ophthalmic drug delivery
4. Rathore KS, Nema RK. Review on ocular inserts. Int J PharmTech Res. 2009 Apr; 1(2):164-9.
5. Ding S. Recent developments in ophthalmic drug delivery. Pharmaceutical Science & Technology Today. 1998 Nov 1; 1(8):328-35.
6. Meisner D, Mezei M. Liposome ocular delivery systems. Advanced drug delivery reviews. 1995 Aug 1; 16(1):75-93.
7. Mohan EC, KanduKuri JM, Allenki V. Preparation and evaluation of in-situ-gels for ocular drug delivery. Journal of Pharmacy Research 2009, 2 (6), 1089. 2009 Jun; 1094.
8. Matrindale. The complete drug reference, 34th ed. Pharmaceutical press. 1996: 127-227.
9. Available at: http://en.wikipedia.org/wiki/Disodium phosphate
10. Eugene F, Fiese F, Timothy ahogen. Preformulation: The theory and practice of industrial pharmacy. 3rded, Verghese publishing House, Bombay, 1987: 171-83.
11. Mohan EC, KanduKuri JM, Allenki V. Preparation and evaluation of in-situ-gels for ocular drug delivery. Journal of Pharmacy Research 2009, 2 (6), 1089. 2009 Jun; 1094.
12. Brahmankar DM, Jaiswal SB. Biopharmaceutics and Pharmacokinetics: Pharmacokinetics. 2nd Ed, Vallabh Prakashan, Delhi: 2009: 399-401.
13. Pharmacopoeia I. Ministry of health and family welfare. Government of India. 1996; 2:350.
14. Available at: www.ICH/stability