EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 1
Issue: 3
104 - 123
Year: 2014

FORMULATION AND EVALUATION OF OPHTHALMIC IN-SITU GELS CONTAINING OFLOXACIN AND DEXOMETHASONE

Tirumaladevi .V, Alekya .T, [1]G.Lakshmana Murthy, K. Sravya, [2]K.Swathi Krishna, [3]C.Roosewelt, [4]P.Kranthi Kumar

[1]Department Of Pharmaceutics, Jagan's College Of Pharmacy, Nellore India.
[2]Department of Pharmaceutics, Saastra college of pharmcy
[3]Department of Pharmaceutical analysis, Jagan's college of pharmacy
[4]Department of Pharmaceutical analysis, Jagan's college of pharmacy

Article Received on 12/10/2014 **Article Revised on 02/11/2014** **Article Accepted on 24/11/2014**

***Correspondence for Author**
Lakshman Murthy
Department Of Pharmaceutics, Jagan's College Of Pharmacy, Nellore India

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 and Dexamethasone. The in-situ gel formulation were prepared by simple mixing method based on the pH triggering system by incorporation of various polymers like HPMC and Carbopol-940 in different proportions. The *in-situ* gel characteristics were evaluated for Clarity, gel pH, gel Capacity, Viscocity,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: Opthalamic*in-situ* gel, Ofloxacin, Dexamethasone,Carbopol-940, HPMC, pH-triggered.

INTRODUCTION

Eye is one of the challenging organ 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, 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 thermoresponsive 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 thermoresponsive 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 and Dexamethasone were obtained as gift samples from MSN labs, Hyderabad. All other chemicals were purchased from Chemspure, Chennai which were of analytical grade.

Table no: 1 Composition of Ophthalmic *Insitu* Gels

INGREDIENTS (gm)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ofloxacin	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Dexamethasone	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol940	0.2	0.2	0.2	0.4	0.4	0.4	0.6	0.6	0.6
Hydroxy propyl methyl cellulose	0.5	1.0	1.5	0.5	1.0	1.5	0.5	1.0	1.5
Citric acid	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407
Disodium hydrogen phosphate	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Distilled water q.s to (ml)	100	100	100	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 dexamethasone was dissolved in acetone separately. The drug solutions were then added to the polymeric solution under constant stirring until an 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.

Simultaneous estimation of Ofloxacin and Dexamethasone: A simple, precise, accurate and reproducible method was developed for simultaneous estimation of Ofloxacin and Dexamethasone. The method involved the measurement of absorptivity data of Ofloxacin and Dexamethasone at 294 nm and 242 nm within beer's range respectively[6]. The method was validated for recovery, repeatability and ruggedness. The quantitative estimation of Ofloxacin and Dexamethasone was carried out using following equations:

$C_x = A_1.Y_2 – A_2.Y_1 / X_1.Y_2 – X_2.Y_1$

$C_y = A_2.X_1 – A1.X_2 / X_1.Y_2 – X2.Y_1$

Table 2 :The absorptivity value of both drugs at 294 nm and 242 nm

DRUGS	At 294nm	At 242mn
Ofloxacin	0.0384 (X_1)	0.0318(X_2)
Dexamethasone	0.0517(Y_1)	0.0537 (Y_2)

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 and Dexamethasone concentration were then determined by simultaneous method at 294 nm and 242 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 Ofloxacinand Dexamethasone 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

suitablely diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 294 nm and 242 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 modes 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.

$\log C = \log C_0 - Kt/2.303$

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 no-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

This model is used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism is not known or more than one type of release phenomenon was involved.

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 was carried out according to the ICH guidelines. Optimized formulation F7 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 no-4: Melting point and Loss on drying of Ofloxacin and Dexamethasone

S.NO	TEST	DRUGS	
		OFLOXACIN	DEXAMETHASONE
1.	Melting point	263ºC	255ºC
2.	Loss on drying	0.15%	0.14%

Analysis of Ofloxacin and Dexamethasone: 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

The IR spectrum of Dexamethasone shown in Fig 11, reveals characteristic peaks in the Dexamethasone IR spectrum that occur at 1202.32 cm-1 for the C-O stretching , 1377.38 cm-1 for the C-F stretching, 2929.44 cm-1 for -C-H stretching , 3761.28 cm-1 for the O-H stretch,free respectively . Fig no-2

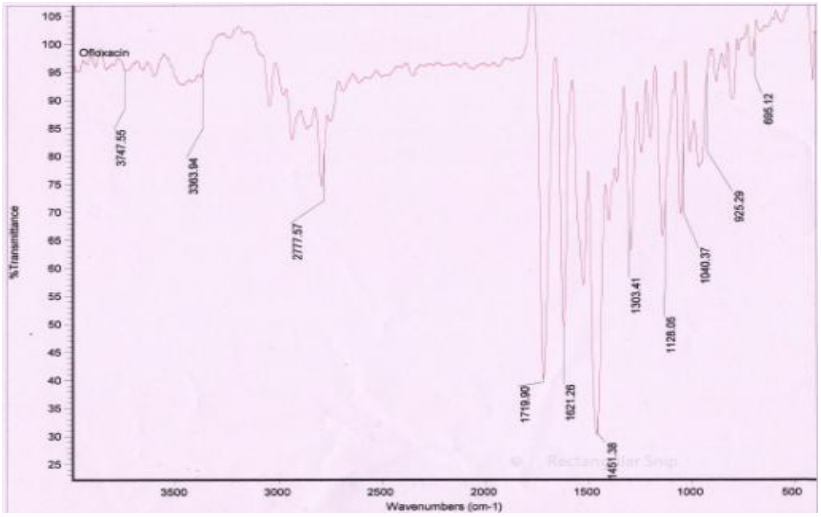


Fig 1: FT-IR spectrum of Ofoloxacin

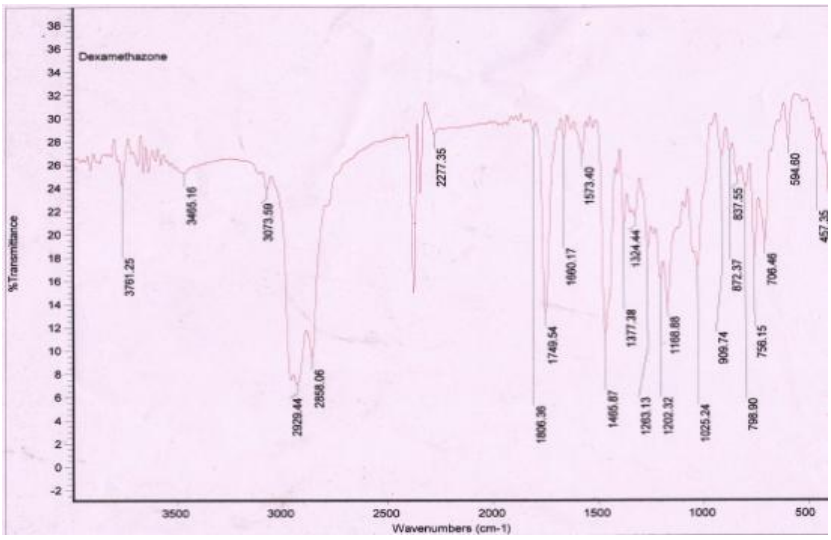


Fig 2: FT-IR spectrum of Dexamethasone

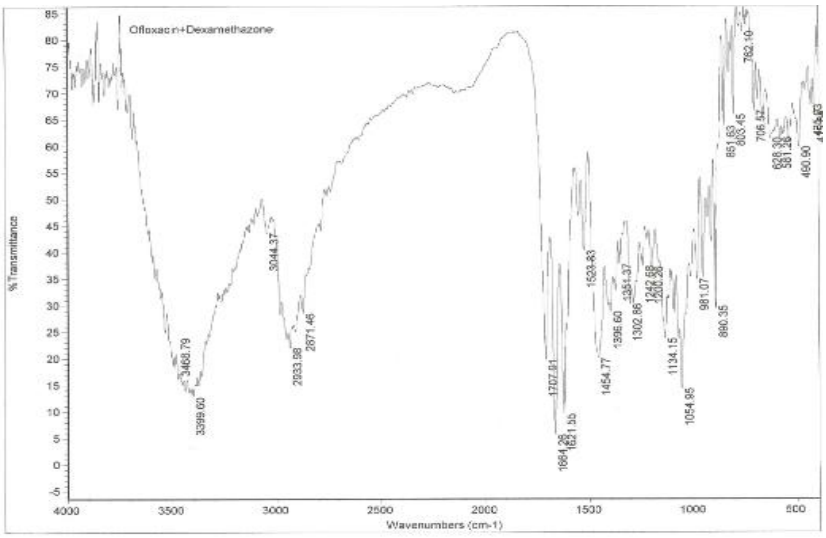


Fig 3:FT-IR spectrum of Ofloxacin and Dexamethasone

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 and Dexamethasone, spectrum of Ofloxacin, and spectrum of Dexamethasone as shown in Fig no3& 4. Therefore it can be concluded that there is no interaction of drugs with excipients.

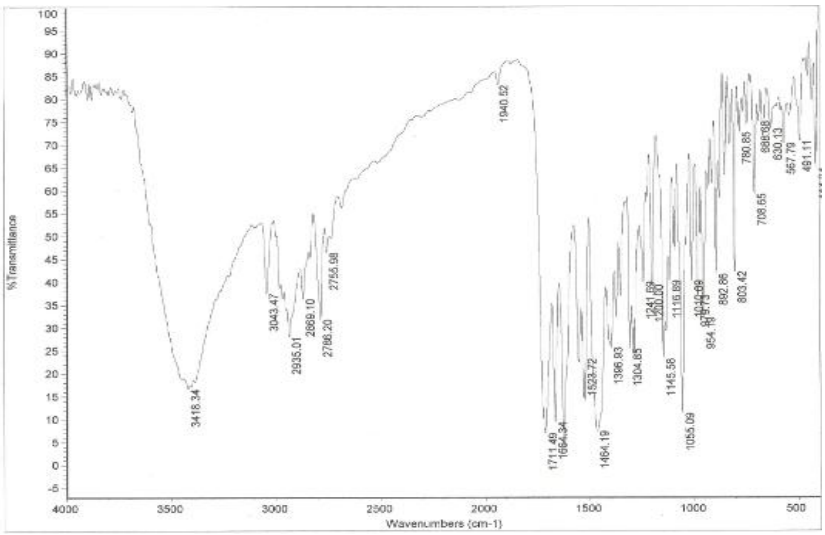


Fig 4: FT-IR spectrum of Physical mixture

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 no-5: Standard curve of Ofloxacin in simulated tear fluid p^H 7.4

Sno	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

STANDARD CALIBRATION CURVE OF DEXAMETHASONE: It was found that the estimation of Dexamethasone by UV Spectrophotometric method at λ_{max} 242.0 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 0.998, at the

concentration range, 1-5 µg/ml. The regression equation generated was y = 0.0731x.(table no-6 & fig no-6)

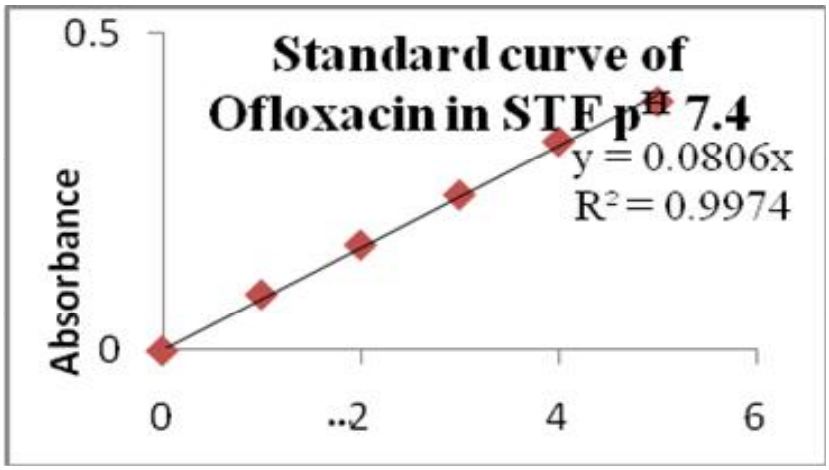


Fig no-5: Standard curve of Ofloxacinin simulated tear fluid p^H 7.4

Table no-6: Standard curve of Dexamethasone in simulated tear fluid p^H 7.4

Sno	Concentration (mcg/ml)	Absorbance at 242 nm
1	1	0.077
2	2	0.151
3	3	0.227
4	4	0.294
5	5	0.357

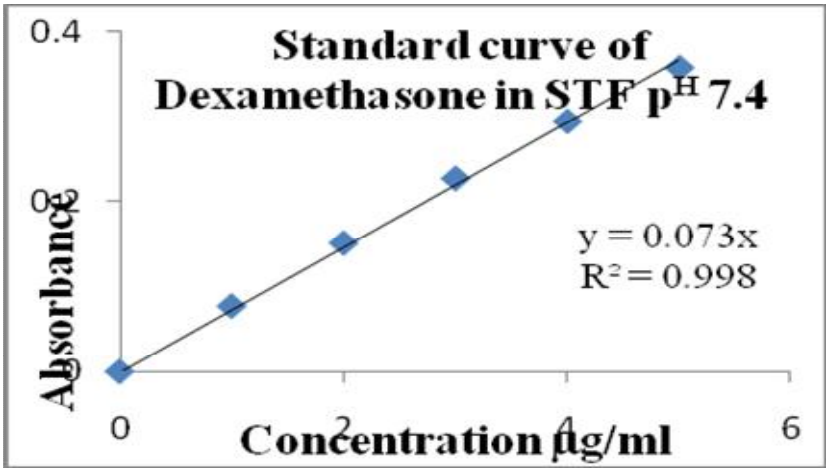


Fig no-6: Standard curve of Dexamethasone in simulated tear fluid p^H 7.4

Formulation of ophthalamic 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 shows 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-F9

S.no	Formulation code	Appearance	Clarity	p^H	Gelling capacity
1	F1	Light yellow	Clear	6.23	-
2	F2	Light yellow	Clear	6.13	+
3	F3	Light yellow	Clear	6.20	+
4	F4	Light yellow	Clear	6.16	++
5	F5	Light yellow	Clear	6.25	++
6	F6	Light yellow	Clear	6.42	+
7	F7	Light yellow	Clear	6.01	+++
8	F8	Light yellow	Clear	5.99	+++
9	F9	Light yellow	Clear	6.08	+++

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

Drug content

The drug content was determined for all formulations and results were shown in table no-8. The percent drug conten was found to be in between 88.33% to 98.66% for Ofloxacin and for Dexamethasone it lies between 86.2% to 96.31 %.

Table 8: Drug content of formulations F1-F9

S.no	Formulation code	% Drug content	
		Ofloxacin	Dexamethasone
1	F1	88.33	86.25
2	F2	89.01	86.71
3	F3	90.66	87.12
4	F4	90.33	89.66
5	F5	91.66	90.26
6	F6	93.33	87.03
7	F7	98.66	96.31
8	F8	93.66	93.56
9	F9	94.05	92.29

Rheological Studies

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

Table 9: Rheological study of formulations F1-F9

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
7	F7	210		184		157
8	F8	262		200		180
9	F9	328		22		195

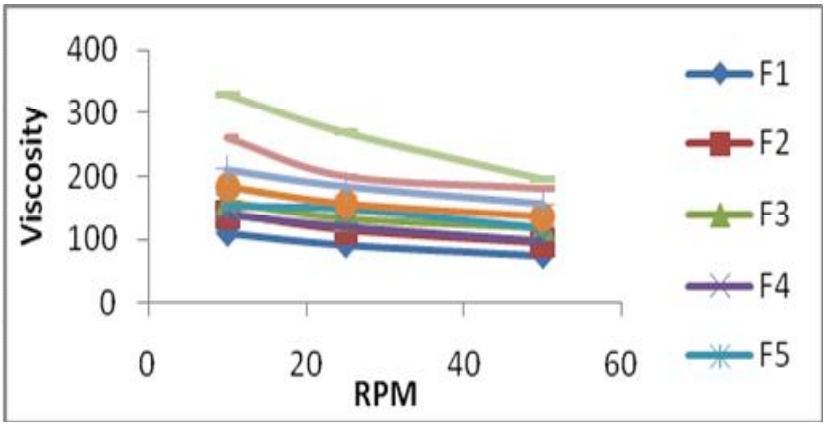


Fig 7: Viscosityof formulations F1- F9 at different RPM

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 were 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% and that of dexamethasone was found to be in between 68.66% to 94.38%. The results were shown in table no-10,11 & fig no- 8 to 11.

COMPARITIVE IN VITRO DIFFUSION STUDY OF DEXAMETHASONE

F1-F9 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)

Overall curve fitting showed that the Dexamethasone release from gels followed Korsmeyer-peppas model except the formulations F5 and F9 which followed Zero order and Higuchi respectively. The critical value of n ranging between 0.652-0.7894 suggests non-Fickian diffusion. (Table no-12)

STABILITY STUDY

The stability studies was 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-13 & 14

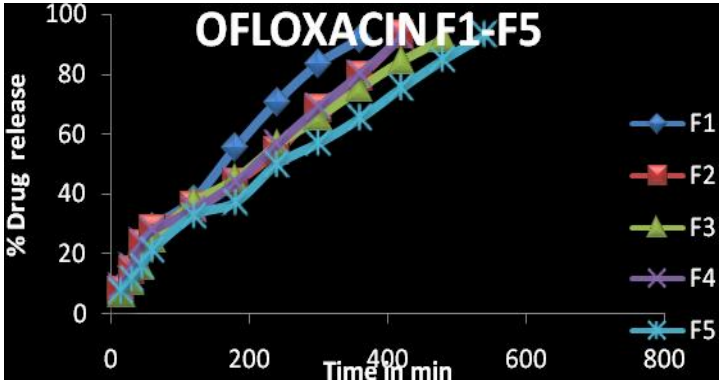
***In vitro* release of Ofloxacin from formulation F1-F9 in STF p^H 7.4 Table no-10**

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	9.31±0.13	7.53±0.55	6.44±0.32	8.86±0.72	7.55±0.15	5.79±0.24	4.47±0.35	4.10±0.47	3.45±0.20
30	15.62±0.34	14.25±0.64	10.63±0.21	15.75±0.09	11.48±0.18	9.62±0.33	8.59±0.22	7.14±0.26	7.89±0.47
45	23.77±0.12	22.80±0.23	15.61±0.24	23.97±0.25	15.72±0.11	14.45±0.76	11.00±0.16	9.33±0.24	10.81±0.32
60	29.13±0.45	27.87±0.12	24.75±0.56	28.03±0.33	21.22±0.21	18.91±0.87	13.23±0.31	12.48±0.51	13.56±0.17
120	38.27±0.63	35.71±0.19	37.17±0.09	34.80±0.11	32.73±0.27	27.62±0.12	25.13±0.12	22.97±0.47	23.49±0.29
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	34.80±0.06	27.29±0.22	29.01±0.84
240	70.70±0.31	54.25±0.21	56.42±0.14	56.67±0.18	49.89±0.65	48.78±0.34	46.89±0.24	33.51±0.16	33.95±0.57
300	83.32±0.35	68.41±0.78	65.61±0.23	69.00±0.29	56.98±0.34	53.66±0.17	53.84±0.15	36.65±0.27	37.58±0.36
360	91.51±0.56	79.18±0.82	75.44±0.18	80.13±0.23	65.37±0.18	62.32±0.12	61.35±0.27	39.17±0.18	45.66±0.33
420		93.06±0.77	84.49±0.43	92.64±0.15	75.57±0.25	70.44±0.23	70.79±0.34	45.81±0.32	49.27±0.76
480			91.78±0.50		84.99±0.02	77.78±0.34	79.72±0.42	50.30±0.35	53.40±0.11
540					93.16±0.32	82.91±0.45	87.90±0.15	58.74±0.14	58.06±0.63
600						87.19±0.62	95.55±0.56	71.65±0.20	62.56±0.42

***Invitro* release of Dexamethasone from formulation F1-F9 in STFp^{H} 7.4 table no-11**

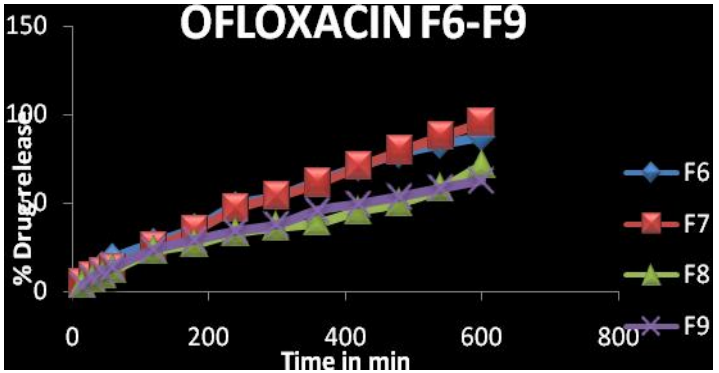
Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
15	8.82±0.12	11.23±0.24	7.14±0.17	8.49±0.52	6.61±0.65	7.58±0.24	5.01±0.09	4.26±0.21	3.67±0.78
30	15.60±0.45	14.74±0.31	14.51±0.23	14.22±0.12	9.03±0.79	9.29±0.56	8.85±0.14	6.59±-.14	6.87±0.57
45	20.04±0.32	17.49±0.33	21.98±0.55	19.26±0.35	11.49±0.12	11.04±0.23	12.02±0.23	8.97±0.22	10.91±0.46
60	25.38±0.67	21.11±0.27	27.52±0.46	22.02±0.32	15.51±0.34	15.16±0.34	15.25±0.35	11.39±0.06	14.25±0.35
120	40.72±0.92	33.00±0.44	36.02±0.51	31.97±0.67	24.18±0.25	25.61±0.45	25.28±0.68	18.35±0.73	23.91±0.52
180	52.23±0.46	46.76±0.35	45.96±0.47	44.49±0.47	33.78±0.12	31.58±0.12	33.26±0.76	26.94±0.02	31.41±0.23
240	65.61±0.23	55.86±0.67	53.39±0.34	55.66±0.78	42.03±0.14	43.13±0.34	42.88±0.34	31.95±0.17	36.70±0.44
300	77.59±0.16	68.41±0.41	65.03±0.44	67.83±0.14	51.96±0.45	49.42±0.45	53.43±0.27	38.55±0.36	42.09±0.67
360	89.78±0.34	79.55±0.31	74.43±0.25	79.43±0.35	63.60±0.36	56.61±0.33	61.93±0.36	43.77±0.24	46.01±0.85
420		91.70±0.46	82.36±0.24	91.24±0.56	71.64±0.67	63.14±0.46	69.84±0.39	46.84±0.67	50.77±0.43
480			91.24±0.32		80.58±0.63	68.99±0.12	78.62±0.26	53.69±0.35	54.83±0.66
540					93.48±0.55	78.07±0.47	86.81±0.17	61.41±0.76	61.30±0.81
600						86.52±0.67	94.38±0.51	70.77±0.89	68.66±0.29

Comparitive in vitro diffusion study of Ofloxacin



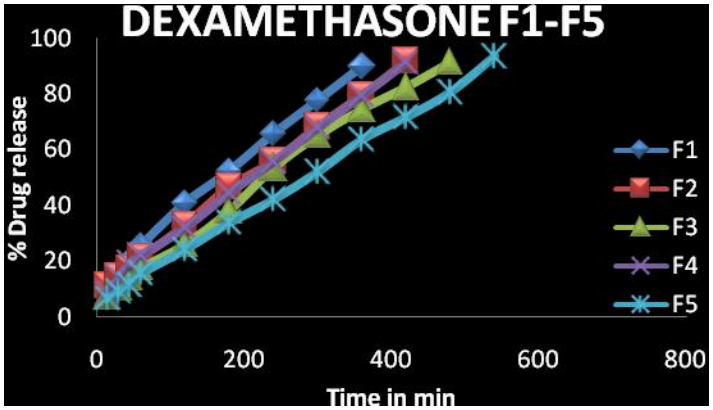
F1-F5 Formulations fig no-8

Comparitive in vitro diffusion study of Ofloxacin



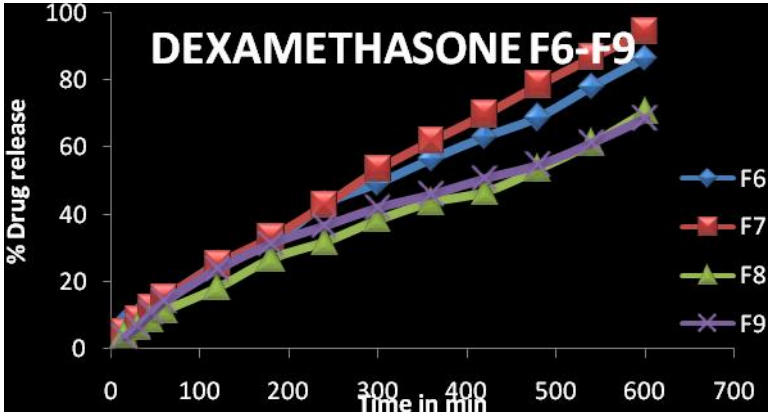
F6-F9 Formulations fig no-9

Comparitive in vitro diffusion study of Dexamethasone



F1-F5 Formulations fig no-10

Comparitive in vitro diffusion study of Dexamethasone



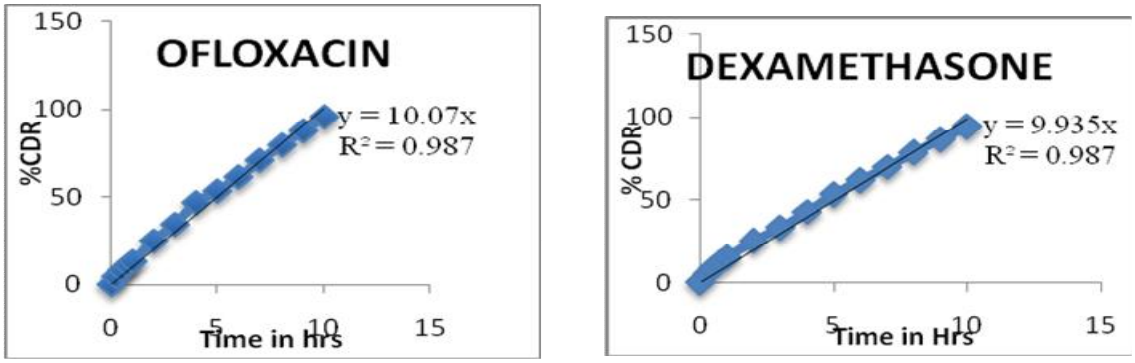
F6-F9 Formulations fig no-11

Drug release kinetics of Formulation F7 table no-12

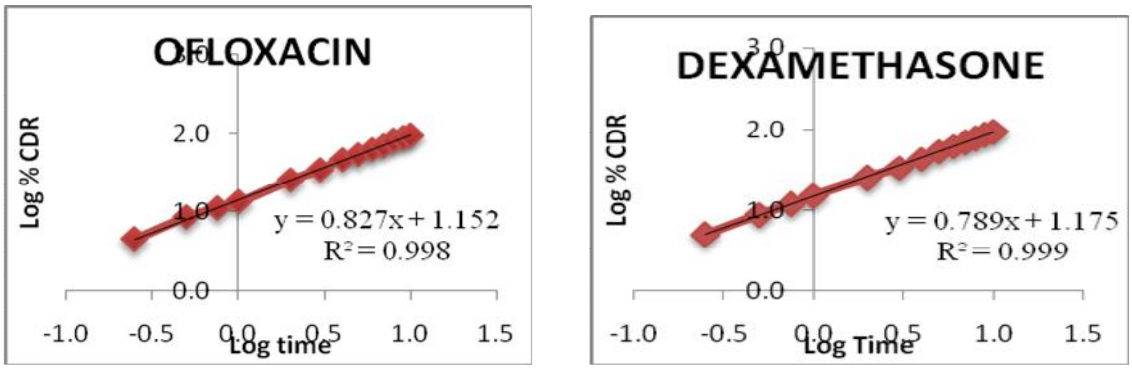
OFLOXACIN & DEXAMETHASONE							
Formulation code	**Zero order R^2**	**First order R^2**	**Higuchi R^2**	**Hixson-crowell R^2**	**Korsmeyerpeppas**		**Best fit model**
					n	**R^2**	
F7	0.9876	0.8890	0.9838	0.9247	0.8274	0.9989	Korsmeyerpeppas
F7	0.9872	0.9559	0.9803	0.9336	0.7894	0.9997	Korsemeyerpeppas

STATISTICAL ANALYSIS: Overall curve fitting showed that the Ofloxacin release from gels followed Korsmeyer-peppas model formulations F7 which followed Higuchi model. The critical value of n ranging 0.8274 suggests non-Fickian diffusion. Table no no-12 & Fig no-12

Overall curve fitting showed that the Dexamethasone release from gels followed Korsmeyer-peppas model the formulations F7 which followed Zero order and Higuchi respectively. The critical value of n ranging 0.7894 suggests non-Fickian diffusion. Table no-12 & Fig no-13



Zero order kinetics of optimized formulaF7: fig no-12



Korsmeyer-Peppas plot of optimized formulaF7 fig no-13

STABILITY STUDY

Table no:13 Physicochemical properties of optimized formulation F7

Time	Appearance	Clarity	Gel p^H	Drug content	
				Ofloxacin	Dexamethasone
Initial	Light yellow	Clear	6.01	98.66	96.31
After 1st Month	Light yellow	Clear	6.00	98.28	96.12

Table no- 14: In vitro drug diffusion studies of optimized formulation F7

Time (hr)	Cum % Drug release			
	Initial		After 1 month	
	Ofloxacin	Dexamethasone	Ofloxacin	Dexamethasone
0.25	4.47±0.35	5.01±0.09	4.03±0.23	5.01±0.11
0.5	8.59±0.22	8.85±0.14	8.36±0.42	8.11±0.24
0.75	11.00±0.16	12.02±0.23	10.54±0.32	12.01±0.45
1	13.23±0.31	15.25±0.35	13.21±0.25	14.49±0.54
2	25.13±0.12	25.28±0.68	24.66±0.12	25.25±0.32
3	34.80±0.06	33.26±0.76	33.87±0.23	33.23±0.46
4	46.89±0.24	42.88±0.34	46.62±0.45	42.85±0.58
5	53.84±0.15	53.43±0.27	53.78±0.37	53.40±0.67
6	61.35±0.27	61.93±0.36	60.85±0.21	61.16±0.26
7	70.79±0.34	69.84±0.39	70.95±0.55	68.29±0.33
8	79.72±0.42	78.62±0.26	78.99±0.76	77.05±0.45
9	87.90±0.15	86.81±0.17	86.94±0.49	85.20±0.27
10	95.55±0.56	94.38±0.51	94.79±0.29	94.24±0.54

CONCLUSION

In the present work, an attempt has been made to develop ophthalmic in-situ gel of Ofloxacin and Dexamethasone and 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 both the drugs.

Simple mixing method that is cooling technique was employed to formulate the opthalamic 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 HPMC (0.5gm) exhibited better results than compared to those other combination of polymers in different concentration. The effectiveness of polymers (Carbopol-940 and HPMC) on the drug release was explained.

The drug release form the optimized formula was found to be diffusion controlled and n value of Peppas equation for Ofloxacin and Dexamethasone were 0.8274 and 0.7844, 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 and Dexamethasone were 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.
2. Wang, NX.; von Recum, HA. "Affinity-Based Drug Delivery". *MacromolBiosci,* 2011;11: 321–332.
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; 1(2): 164-169.
5. Ding S. Recent developments in ophthalmic drug delivery. Pharm SciTechnol Today, 1998; 1: 328–335.
6. Meisner D, Mezei M. Liposome ocular delivery systems. AdvDrug Deliv Rev, 1995; 16:75–93.
7. Mohan EC, Jagan Mohan K, Venkatesham A. Preparation and evaluation of insitu gels for occular drug delivery. J Pharm Res 2009;2(6):1089-94.

8. Matrindale. The complete drug reference, 34th ed. Pharmaceutical press. 1996:127-227.
9. 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, E.C., Kandukuri, J.M. Allenki, V. Preparation and Evaluation of In-Situ-Gels for Ocular Drug Delivery, Journal of Pharmacy Research. 2009; 2(6): 1089-1094.
12. Brahmankar DM, Jaiswal SB. Biopharmaceutics and Pharmacokinetics: Pharmacokinetics. 2nd ed. VallabhPrakashan, Delhi: 2009; 399-401.
13. Controller of Publication. Indian Pharmacopoeia, Vol. 2, Ministry of Health and Family Welfare, Government of India, New Delhi, pp: A117- A147.
14. www.ICH/stability