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DESIGN AND CHARACTERIZATION OF PH-TRIGERED INSITU GEL CONTAINING GATIFLOXACIN AND DEXOMETHASONE BY 3^2 FACTORIAL DESIGNS

Sravanthi Reddy Emmadi*, S. A. Sreenivas

*Research Scholar, Mewar University, Gangrar, Chittorgarh, Rajasthan, India
Sree Datta Institute of Pharmacy, Hyderabad

Corresponding author E-mail: sravzreddy.80@gmail.com*

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ABSTRACT

A major problem in ocular therapeutics is the attainment of optimal drug concentration at the site of action, which is compromised mainly due to precorneal loss resulting in only a small fraction of the drug being ocularly absorbed. The effective dose administered may be altered by increasing the retention time of medication into the eye by using in situ gel forming systems. The aim of the present investigation is to prepare and evaluate novel in situ gallen gum based ophthalmic drug delivery system of Gatifloxacin and dexamethasone. All the formulations were sterilized in an autoclave at 121°C for 15mins. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, in vitro diffusion study and ICH stability studies. The developed formulations exhibited sustained release of drug over a period of 7 hours thus increasing residence time of the drug and optimized formulations also found satisfactorily stable, thus these in situ gelling systems may be a valuable alternative to the conventional systems.

INTRODUCTION

The eye is a complex and unique part of the human organs that has been considered as the window to the human soul. Broadly, the human eye is divided into two segments that are anterior and posterior segments . The specific dis-ease conditions of the eye are associated with each of these broad segments. For instance, conjunctivitis, glaucoma, ble-pharitis, and cataract are some of the diseases that affect the anterior segment of the eye, while diabetic retinopathy and age-related macular degeneration are known to affect the pos-terior segment.[1] Due to the unique structure of the eye, which inhibits the entry of drug molecules into the desired site, the ophthalmic delivery of the drug has been one of the most challenging tasks for a pharmaceutical scientist. Eye drops accounts for more than 90% of ophthalmic preparations on the markets. However, they are washed away from the eye and results in low ocular bioavailability (< 5%) after topical administra-tion [3] by different elimination mechanisms[2]. This elimination process includes tear turnover, nasolacrimal drainage, pro-tein binding, systemic absorption, enzymatic degradation and complex penetration barriers (Corneal Barrier, Blood Aqueous Barrier (BAB), and Blood Retinal Barrier

(BRB)). The scope of the present work is to formulate a ph sensitive *in-situ* gel for ocular delivery. For local ophthalmic delivery system Gatifloxacin – a potent antibacterial agent and Dexamethasone – a steroid is used for controlled release. This formulation follows zero order kinetics – peppas model. Conventional delivery system often results in poor availability & therapeutic response because high tear fluid turns over & dynamics which cause quick elimination of the drug from the eye so, to conquer the bioavailability problem ophthalmic *in-situ* gel is developed. To improve the bioavailability viscosity enhancers such as HPMC K_4M/ carbopol 934P/gellan gum are used to increase the viscosity of formulation in order to prolong the pre-corneal residence time & improve the bioavailability, ease to manufacture. Penetration enhancer: preservatives, chelating agent, surfactants are used to enhance corneal drug penetration[3.] Ophthalmic in-situ gelling is comprising of environmentally sensitive polymers that will be altered structurally with the small changes in specific conditions like pH, temperature and ionic strength in the environment. In-situ forming gels are liq- uids during instillation into the eye and then undergoes rapid gelation in the cul-de-sac of the eye to form viscoelastic gels in response to environmental changes. Attempt has been made to use blend of polymers in design of sustained ocular delivery system of Gatifloxacin and Dexamethasone. Study was mainly focused on investigating influence of Carbopol 940 and HPMC K15 M polymers and their concentration on ocular delivery using factorial design statistically.

MATERIAL AND METHOD:

Gatifloxacin (1-Cyclopropyl-1,4-dihydro-6-fluoro-8-methoxy-7-(3-methyl-1-piperazinyl) -4- oxo- 3-quinolinecarboxylic acid) and Dexamethasone [8S, 9R, 10S, 11S, 13S, 14S, 16R, 17R - 9 - fluoro – 11 – 17 - dihydroxy – 17 - (2-hydroxyacetyl) - 10,13,16- trimethyl 6,7,8,9,10,11,12,13,14,15, 16, 17-dodecahydro - 3H - cyclopentane phenanthrene-3-one] obtain as gift sample from Redchem lab Mumbai. Gellan gum obtain as gift sample Applied bioscience Mumbai. All other chemical used either AR/LR Grade.

Drug-excipients compatibility study: Infrared spectra matching approach was used for detection of any possible chemical interaction between the drug and excipients. The drug, 1:1 physical mixture of cholesterol/drug/span/tween (each 10 mg) was mixed with 400 mg of potassium bromide. About 100 mg of this mixture was compressed to form a pellet using a hydraulic press at 10 tones pressure. Pellets were scanned in the range of 4000-400 cm-1 in FTIR spectrophotometer (FT/IR-4100 type A spectrophotometer, Jasco, Japan). The IR spectrum of physical mixtures was compared with that of pure drug to detect any appearance or disappearance of peaks[5].

Differential scanning calorimetry (DSC): Differential scanning calorimetry (DSC) scans of pure drug and drug loaded insitu gel were performed using DSC 1/700 (Mettler Toledo, Germany). The analysis was performed with a heating range of -25 ^{0} C to 250 ^{0} C and at a rate of 10 °C/min in nitrogen atmosphere. The sample weight was approximately 6 mg

Preliminary screening: Polymers were selected from Carbopol 934, Carbopol 940, HPMC K4 M and Gellan Gum based on the type of gel formed at different concentrations, the strength of the in-situgel formed and appearance of the gel. Bearing in mind all these factors, it was concluded that PVP and PEO with PMMA form very good insitu gel

Selection of polymers: Polymers were selected from among PVP, PEO, HPMC, EC and PMMA based on the type of in situgel formed at different concentrations, the strength of the films formed and appearance of the films. Bearing in mind

all these factors, it was concluded that PVP and PEO with PMMA form very good films

Design of experiment: A 3-level 2-factor full factorial design was implemented for the formulation and optimization of insitu gel. This design is suitable for exploring quadratic response surface and constructing second order polynomial models. The non linear quadratic model generated by the design in the form:

$$Y = a_0 + b_1 X1 + b_2 X2 + b_3 X1^2 + b_4 X2^2 + b_5 X1X2 + E$$

Where, Y is the measured response associated with each factor level combination: a_0 an intercept: b_1-b_5 are the regression coefficients: X1 and X2 are the independent factors studied and E is the associated error term. The amount of Carbopol 940 (X1) and amount of HPMC K15M (15cps) (X2) were selected as independent variables. Furthermore, viscosity, gelation capacity drug content and invitro drug release were selected as dependent variables Independent factors used in the design are enlisted in Tables X and XX show applied 3^2 full factorial design[6]

Preparation of Insitu gel: Cold method was used to prepare dorzolamide hydrochloride in situ gel . Half of the desired volume of distilled water, containing accurately weighed HPMC K15 M, was kept in refrigerator (for cooling) to get a clear solution. The desired amount of Carbopol 940 as reffered table was added in HPMC K15 M solution with continuous stirring and solutionwas stored at 4 ^{0}C to obtain clear solution. The solution of desired amount of drug (0.5% w/v), sodium chloride (0.5% w/v) and benzalkonium chloride (0.01% v/v) was prepared in distilled water. This drug, sodium chloride and benzalkonium chloride solution mixed with polymeric solution under constant stirring to get clear solution.

Evaluation and statistical optimization

Physicochemical evaluation/characterization of ocular insitu gel:

Physical appearance

Clarity and pH: The clarity was determined by using visual inspection under black and white background. The formulation pH was determined by using pH meter (Equiptronics, Model EQ-610).

Rheological study: Viscosity of formulations was measured by using small volume adapter of Brookfield Viscometer (DV-II þ Pro, Brookfield Engineering Labs. Inc., Middle boro, USA) equipped with helipath stand and T bar spindle. Viscosity measurements were made at nonephysiological (25 ^{0}C) and physiological (37 ^{0}C) temperature. Measurements were done in triplicate with varying the rotation speed of spindle from 5 to 150 rpm.

Gelling capacity: *In-vitro* gelling capacity was determined by visual method. For assessing gelation capacity, 100 µL of prepared formulations(F1 to F9) was transferred in a glass test tube containing 2 mL of simulated tears fluid (STF)freshly prepared (pH 7.2)and equilibrated at 37±1oCtemperature. The tubes were gently shaken for manually simulation of eye blinking and to observe gel dissolution. The gel formation was visually assessed. Elapse time in sol to gel and gel to sol was noted. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which the formed gel remains as such. The composition of STF used for this study was as follow: sodium chloride (0.670g), sodium bicarbonate (0.200g), calcium chloride·2H2O (0.008g), and purified water q.s. (100g). The in-vitro gelling capacity was graded in three categories on the basis of gelation time and time period for which the formed gel remains as such. The lowest scores (+) were assigned to those formulations in which the phase transition Occurred only after 60–90 s and the formed gels collapsed within 1–2 h. The highest scores

(+++) were assigned to those products in which the phase transition commenced within 60–90 s and the gels so formed were stable for about 7–8 h. The moderate scores (++) were assigned to the products, which formed the gel in 60–90 s but failed to maintain gel structure for more than 4 h.

Bioadhesive Strength: "Detachment Stress is the force required to detach the two surfaces of mucosa when a formulation/gel is placed in between them". The detachment stress was measured by using a modified analytical balance (A). A fresh goat membrane was obtained from local slaughter house7. A section of fresh mucosa was cut from the goat eye and washed with saline solution.[7]

Drug content: The concentration of Gatifloxacin(GAF) and Dexamethasone(DM) present in ophthalmic gel was determined by simultaneous method as reported in **Kumar *et al.*, 2011** with slight modification. The weight quantity of formulation was diluted with 100 mL PBS (pH6.8) and resulting solutions were filtered through 0.45 µm membrane filters. The samples were suitably diluted and analyzed spectrophotometrically. A simultaneous estimation method for GAF and DM at 286 nm and 242 nm, respectively was employed using UV-visible spectrophotometer (UV-3200 Pharmaspec, Labindia) [8]

Sterility studies: Sterility is one of the most vital requirements for an ophthalmic preparation to determine the absence of viable microorganisms that may harm the eye of the patient. FTM and SCDM were used for determining the presence/absence of aerobic/anaerobic bacteria and fungus. Microbial growth was observed in FTM and SCDM media which were inoculated with positive control on the 2nd day. No growth was observed in sterilized ophthalmic *in situ* gels. Based on these observations (Tables XXXX,), it was concluded that moist heat sterilization of polymeric solutions and drug sterilization by filtration followed by aseptic crystallization could be done to achieve the sterility.[9]

Isotonicity Evaluation: For the study of isotonicity formulations were mixed with few drops of diluted blood on a slide. The diluted blood was re ared by using Grower's solution and Slide was observed under microscope at 45x magnification12,13. The shape of blood cells were compared with standard marketed ophthalmic formulation

In-vitro release studies: *In-vitro* release studies can help in investigating mechanisms behind skin permeation of the drug. Modified Franz diffusion cell was used to determine *in-vitro* drug release profile of OFL and DS concurrentlyin15mL of STF at pH 7.4 which was added to the acceptor chamber. The temperature within the chamber was maintained at 37±0.5 ^{0}C. The dissolution medium was stirred with magnetic bead at 50 rpm using magnetic stirrer, to prevent the formation of concentrated drug solution layer below the standard membrane. At predetermined time intervals, 1mL of sample was withdrawn from the acceptor compartment and replaced the sample volume with TDW. The samples were diluted with STF pH 7.4, filtered and the amount of drug release was analyzed in UV at respective wavelength.[10]

Kinetics of Drug Release: Zero order release (Eq. (1)), Higuchi (Eq. (2)), and first order (Eq. (3)), as well as Korsmeyer-Peppas model (Eq. (4)) release kinetic models were applied to process the in-vitro release data of optimized formulation to find mechanism behind the drug release form the developed system.

$Q = k1t$ …….....eq.1

$Q = k2 (t) 0.5$…….....eq.2

$Q = 100(1 - e{-}k3t)$ …….eq.3

__/__=____ ……eq.4

Where Q is the percentage release at time t. k1, k2 and k3 are the rate constants of zero order, Higuchi, and first order model, respectively. Whereas, Mt/Mα is the fraction of the drug release at time t, Kp is

the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms and is calculated from the slope of the plot of log of fraction of drug released (Mt / Mα) vs log of time (t)[24].

Antimicrobial activity: The results of the antimicrobial efficiency of developed formulation F8 (test) and OFL solution (standard, 0.3%w/v), diluted with PBS; pH 6.4 are shown in **Figure XXX**. The results of the antimicrobial efficiency test revealed that there was no significant change in the antimicrobial activity of drug due to formulation ingredients and conditions, when compared with GFL standard solution. Antimicrobial efficacy of tested formulation was found to be increase with increased drug concentration. Developed formulation showed ≥86% antimicrobial efficacy against *Staphylococcus aureus* compared with standard OFL solution

***In vivo* studies**

Eye irritation test: The possibility of eye irritation due to the *in situ* gel administration was evaluated in rabbits. The ocular safety observation for the formulated ophthalmic *in situ* gels in the rabbit eyes adopting Draize's scoring approach. The ocular safety score for the formulated ophthalmic i*n situ* gel was found to be 0.33 and therefore, all formulations considered as practically non-irritating to rabbits eye. These safety scores are very insignificant when compared to the maximum score of 110. Three veterinarians independently graded the rabbits for ocular lesions and no symptoms of ocular irritation (Table 7.39) such as redness, tearing, inflammation or swelling were observed after *in situ* gel administration.

Corneal Residence Evaluation: Poor ocular bioavailability of conventional ophthalmic dosage forms is attributed to certain precorneal constraints, namely, solution drainage, lacrimation, tear dilution, tear turnover (about 16 %) and conjunctival absorption. Binding of drug to protein also contributes to the loss of drug through the precorneal parallel elimination loss pathway. The tears contain both bound and free drug to overcome the limitations of conventional therapy and achieve optimum efficacy. Precorneal residence time of ocular drugs is being assessed by certain invasive techniques (Lee, 1993) and non-invasive techniques (Greaves *et al.*, 1991)

Stability studies: One of the major criteria for any rational design of a dosage form is its stability. Drug instability in pharmaceutical formulations may be detected in some instances by a change in the physical appearance, colour, odor, taste, or texture of the formulation. Whereas, in other cases, chemical changes may occur which are not self evident and may only be ascertained through chemical analysis. Scientific data pertaining to the stability of a formulation leads to the prediction of the expected shelf life of the proposed product and when necessary, to the reformulation of the dosage form. Ideal batches of the formulated sterile ophthalmic *in situ* gels in sterile plastic eye drop bottles. Stability studies were carried out for 6 months at room temperature 20°C ± 5°C. It was observed that there was no significant change in terms of physical appearance, drug particle size, drug content and gelling properties after six months of storage at room temperature.

RESULTS AND DISCUSSION

Clarity and pH: Clarity of all formulations was found to be satisfactory. The pH of the formulation was found in the range 6.8 -7.2 Formulation with wide pH range 3.5 to7.5 can be applied on eye without any problem

Bio adhesive Strength: The detachment stress of formulation is shown in Table XX Bioadhesive force means the force with which gels bind to ocular mucosa. Greater bioadhesion is indicative of prolonged residence time of a gel and thus prevents its drainage from cul-de-sac. The bioadhesion force increased significantly

as the concentration of bioadhesion polymers increased.

Figure: 1

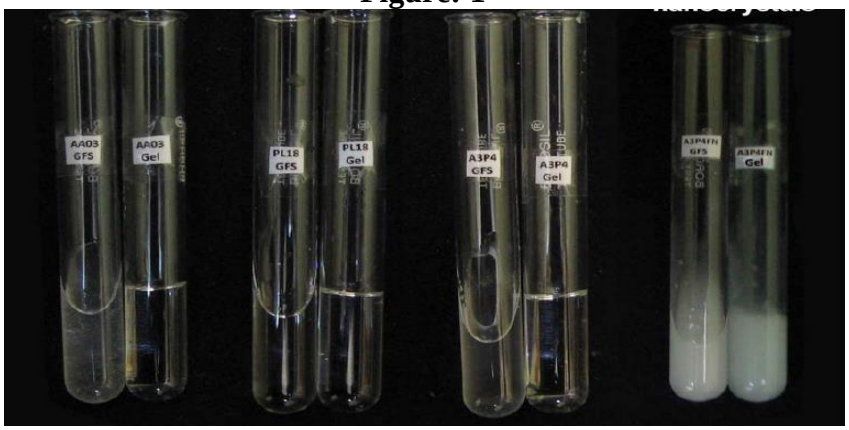


Figure: 2 FTIR of pure gatifloxacin

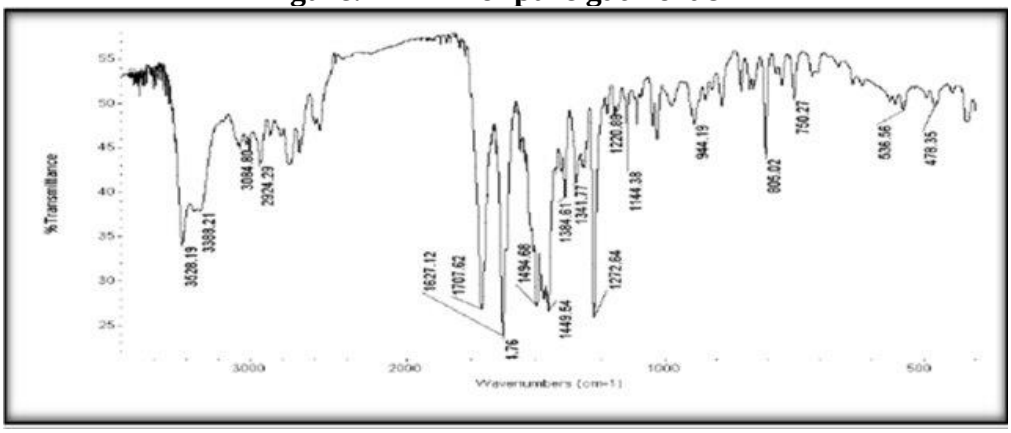


Figure: 3 FTIR of pure dexamethasone

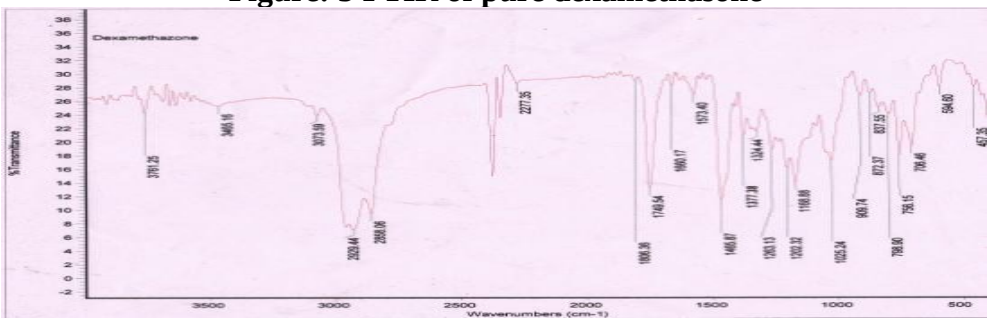


Figure:4 FTIR OF Carbopol

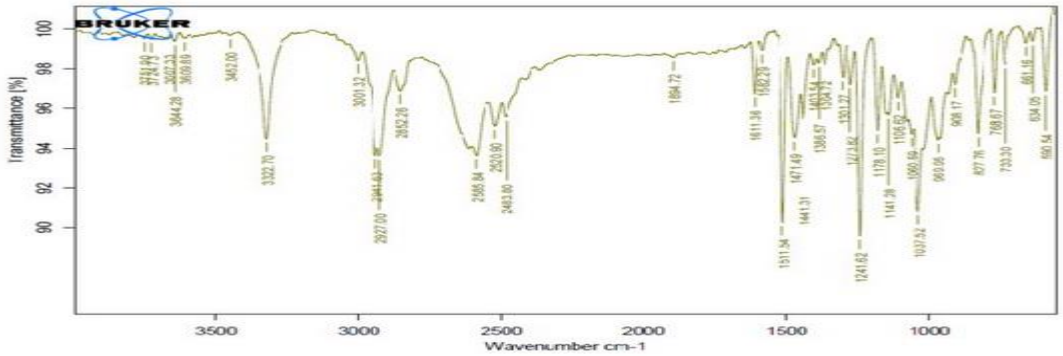


Figure:5 FTIR OF Physical Mixture

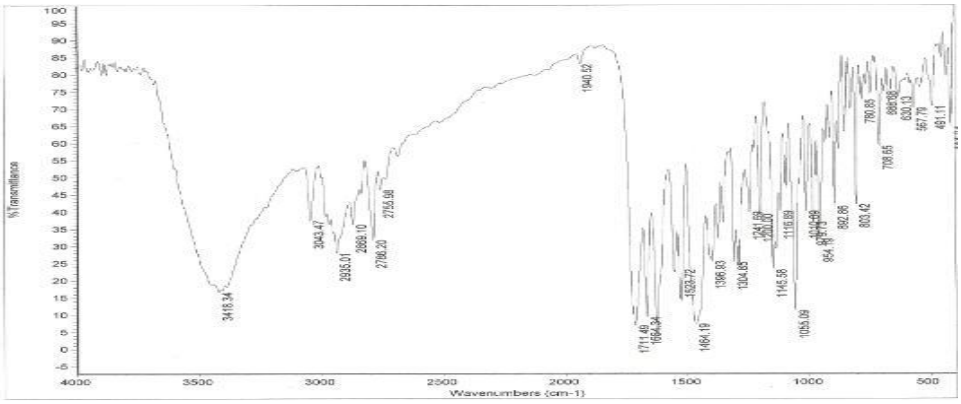


Figure:6 DSC of pure gatifloxacin

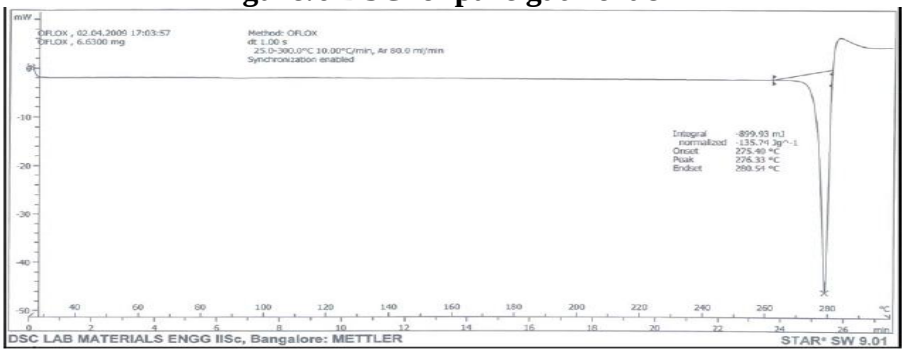


Figure:7 DSC of pure dexamethasone

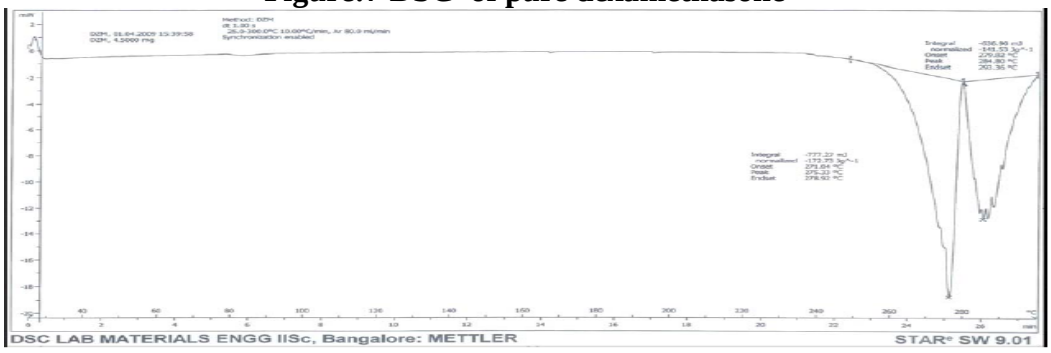


Table: 3 Gelling Capacity and Sol-Gel Temperture

Sl no	Formulation code	Gelation Tempreture	Clarity	pH	Gelling Capacity
1	F1	38.23±0.20	Clear	6.23	++
2	F2	31.33±0.21	Clear	5.99	++
3	F3	36.23±0.10	Clear	6.22	+++
4	F4	39.44±0.21	Clear	6.19	+++
5	F5	38.23±0.20	Clear	6.32	+++
6	F6	36.44±0.10	Clear	6.02	+++
7	F7	30.71±0.30	Clear	6.21	+++
8	F8	37.23±0.10	Clear	6.12	+++
9	F9	31.26±0.11	Clear	6.31	+++
(+)gels after few minutes dissolves rapidly,(++) gelation immediate remains for few hour (+++)gelation immediate remains for extended period (n=3 ,Mean±SD)					

Table:4 Bioadhesive Strength

Sl no	Formulation	Deattachment Force(N)
1	F1	0.1898±0.03
2	F2	0.2019±0.02
3	F3	0.2424±0.01
4	F4	0.3513±0.06
5	F5	0.4398±0.03
6	F6	0.6407±0.04
7	F7	0.5314±0.19
8	F8	0.7843±0.08
9	F9	0.4567±0.13
n=3 Mean±SD		

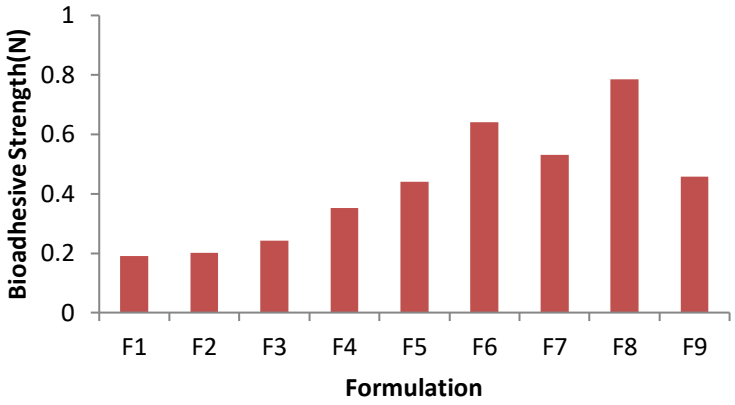
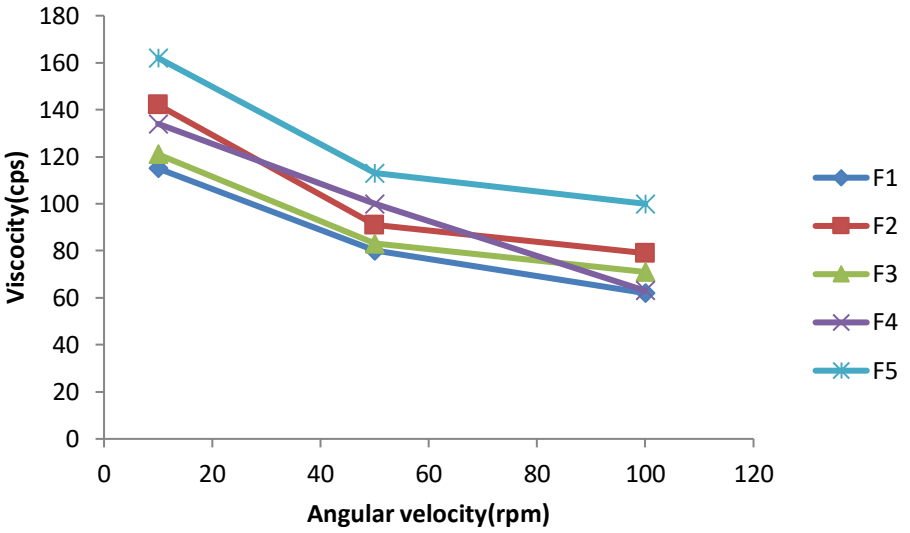


Table: 5 Drug Content

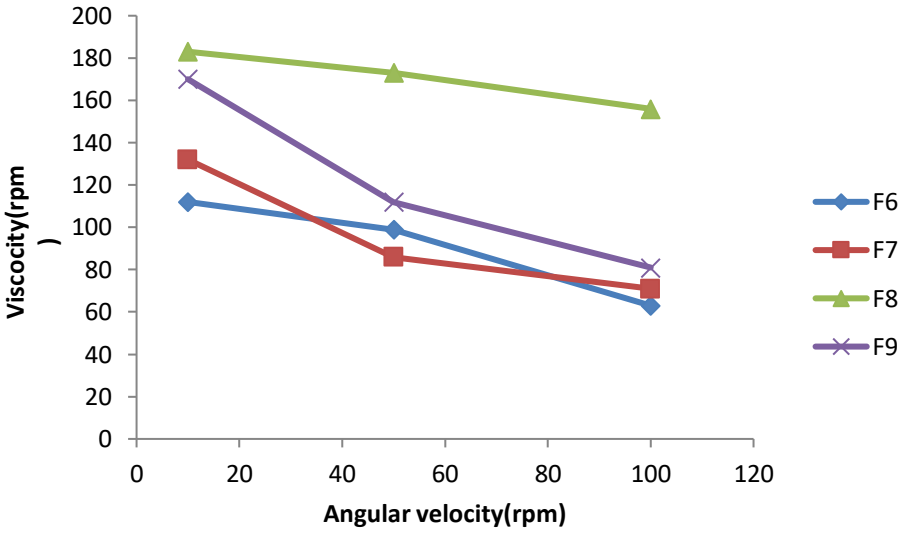
Sl no	Formulation	% of Drug content	
		Gatifloxacin	Dexamethasone
1	F1	97.08±0.06	90.17±0.06
2	F2	96.18±0.04	92.17±0.05
3	F3	97.08±0.06	93.27±0.16
4	F4	97.08±0.06	91.43±0.08
5	F5	99.07±0.36	93.27±0.36
6	F6	100.05±0.16	90.17±0.06
7	F7	97.08±0.06	90.67±0.06
8	F8	101.13±0.05	99.23±0.16
9	F9	97.08±0.06	91.10±0.26
n=3 , Mean±SD			

Table:6 Rheological Study for all insitu gel Formulation

Sl no	Formulation	Viscosity at different rpm(cps)		
		10rpm	50rpm	100rpm
1	F1	115	80	62
2	F2	142	91	79
3	F3	121	83	71
4	F4	134	100	63
5	F5	162	113	100
6	F6	112	99	63
7	F7	132	86	71
8	F8	183	173	156
9	F9	170	112	81



Rheograph of F1-F5



Rheograph of F6-F9

Table:7 Sterility Results in FTM medium for pH-triggered *in situ* gel

Test group	Sample	Day of sterility testing and Observations (32.5^0C ± 2.5^0C)							
		1	2	3	4	5	6	7	14
Control	Positive	+	+	+	+	+	+	+	+
	Negative	-	-	-	-	-	-	-	-
Sterilised Sample	F1	-	-	-	-	-	-	-	-
	F2	-	-	-	-	-	-	-	-
	F3	-	-	-	-	-	-	-	-
	F4	-	-	-	-	-	-	-	-
	F5	-	-	-	-	-	-	-	-
	F6	-	-	-	-	-	-	-	-
	F7	-	-	-	-	-	-	-	-
	F8	-	-	-	-	-	-	-	-
	F9	-	-	-	-	-	-	-	-

(-)Absence of Microbial (Bacteria) growth (+) Presense of Microbial (Bacteria) growth

Table: 8 Sterility Results in SCDM medium for pH-triggered *in situ* gel

Test group	Sample	Day of sterility testing and Observations (32.5^0C ± 2.5^0C)							
		1	2	3	4	5	6	7	14
Control	Positive	+	+	+	+	+	+	+	+
	Negative	-	-	-	-	-	-	-	-
Sterilised Sample	F1	-	-	-	-	-	-	-	-
	F2	-	-	-	-	-	-	-	-
	F3	-	-	-	-	-	-	-	-
	F4	-	-	-	-	-	-	-	-
	F5	-	-	-	-	-	-	-	-
	F6	-	-	-	-	-	-	-	-
	F7	-	-	-	-	-	-	-	-
	F8	-	-	-	-	-	-	-	-
	F9	-	-	-	-	-	-	-	-

(-)Absence of Microbial (Bacteria) growth (+)Presense of Microbial(Bacteria)growth

Table: 9 Sterility Results in FTM medium for pH-triggered *in situ* gel

Test group	Sample	Day of sterility testing and Observations (22.5^0C ± 2.5^0C)							
		1	2	3	4	5	6	7	14
Control	Positive	+	+	+	+	+	+	+	+
	Negative	-	-	-	-	-	-	-	-
Sterilized Sample	F1	-	-	-	-	-	-	-	-
	F2	-	-	-	-	-	-	-	-
	F3	-	-	-	-	-	-	-	-
	F4	-	-	-	-	-	-	-	-
	F5	-	-	-	-	-	-	-	-
	F6	-	-	-	-	-	-	-	-
	F7	-	-	-	-	-	-	-	-
	F8	-	-	-	-	-	-	-	-
	F9	-	-	-	-	-	-	-	-

(-)Absence of Microbial (fungal) growth (+) Presense of Microbial (fungal) growth

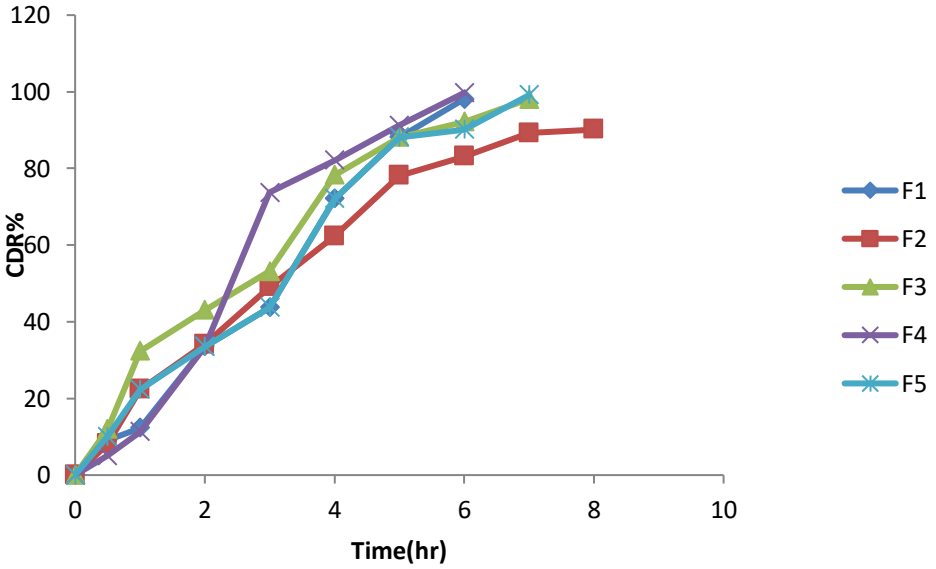
Table: 10 Sterility Results in SCDM medium for pH-triggered *in situ* gel

Test group	Sample	Day of sterility testing and Observations (22.5^0C ± 2.5^0C)							
		1	2	3	4	5	6	7	14
Control	Positive	+	+	+	+	+	+	+	+
	Negative	-	-	-	-	-	-	-	-
Sterilised Sample	F1	-	-	-	-	-	-	-	-
	F2	-	-	-	-	-	-	-	-
	F3	-	-	-	-	-	-	-	-
	F4	-	-	-	-	-	-	-	-
	F5	-	-	-	-	-	-	-	-
	F6	-	-	-	-	-	-	-	-
	F7	-	-	-	-	-	-	-	-
	F8	-	-	-	-	-	-	-	-
	F9	-	-	-	-	-	-	-	-

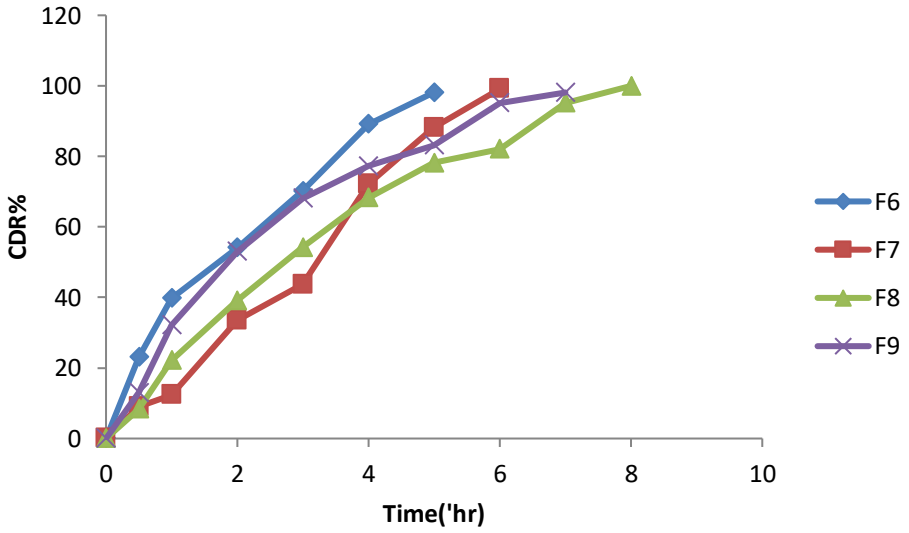
(-)Absence of Microbial(fungal)growth (+)Presense of Microbial(fungal)growth

Table:11 In vitro Release Study of Gatifloxacin inSTF 7.4 pH

Time (hr)	FORMULATION - % of Drug Release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	9.01	8.11	11.98	5.02	10.01	23.11	9.01	8.32	12.98
1	12.34	22.34	32.34	11.34	22.34	39.77	12.34	22.14	32.14
2	33.54	34.14	43.04	33.54	33.54	54.11	33.54	39.12	53.04
3	43.71	49.01	53.11	73.71	43.71	70.21	43.71	54.21	68.11
4	72.14	62.33	78.24	82.11	72.14	89.11	72.14	68.26	77.24
5	88.13	78.13	88.13	91.23	88.13	98.16	88.13	78.13	83.13
6	98.11	83.11	92.11	99.72	90.11	-	99.11	82.03	95.11
7	-	89.23	98.11	-	99.13	-	-	95.11	98.11
8	-	90.11		-		-		99.99	



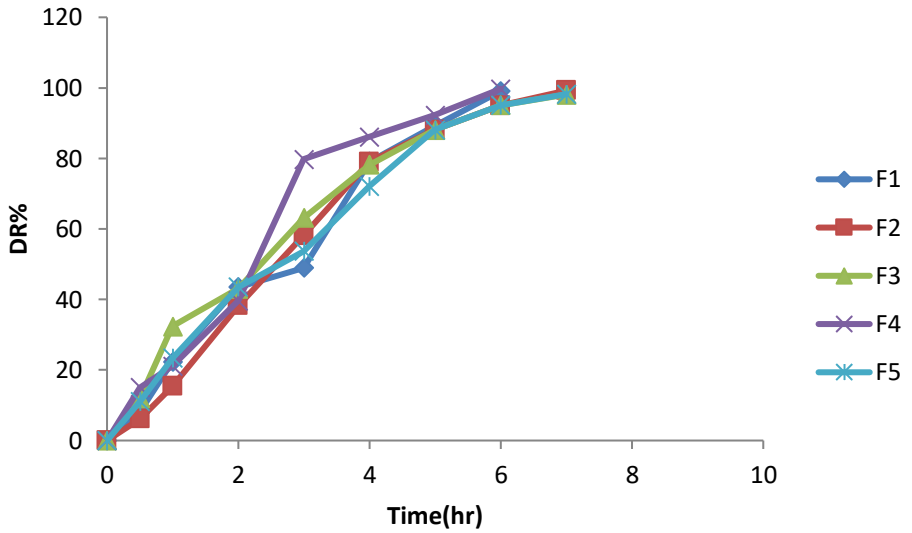
Gatifloxacin Drug Release Profile for F1-F5 inSTF 7.4 pH



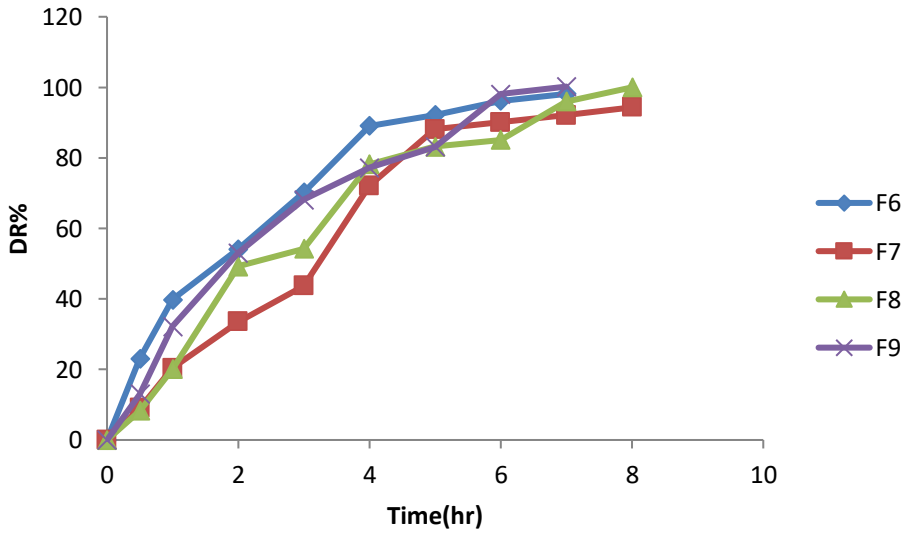
Gatifloxacin drug release Profile for F6-F9 inSTF 7.4 pH

Table:12 *In vitro* Release Study of Dexamethason inSTF 7.4 pH

Time (hr)	FORMULATION (% of Drug Release)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	8.01±0.03	6.12	11.98	15.02	11.01	23.11	9.01	8.32	12.98
1	22.34±0.04	15.34	32.34	21.14	23.34	39.77	20.34	20.14	32.14
2	43.54±0.01	38.24	43.04	39.64	43.54	54.11	33.54	49.22	53.04
3	49.01	58.11	63.11	79.72	53.70	70.21	43.71	54.21	68.11
4	79.11	79.03	78.24	86.11	72.14	89.11	72.14	78.20	77.24
5	89.23	88.13	88.13	92.23	88.13	92.16	88.13	83.23	83.13
6	99.10	95.01	95.10	99.72	95.11	96.13	90.11	85.13	98.11
7	-	99.23	98.11	-	98.23	98.13	92.12	96.01	100.21
8	-	-		-		-	94.31	99.99	



Dexamethasone drug release Profile for F1-F5 inSTF 7.4 pH



Dexamethasone Drug Release Profile for F6-F9 inSTF 7.4 pH

***In-vivo* Study Eye irritation test**

Table: 13- Ocular Safety score for insitu gel in Rabbit eyes

Group	Day	Mean Recorded score						Safety score						Total score	Safety Rating
		Cornea	iris			Conjunctiva		Cornea	iris			Conjunctiva			
		O	A	I	R	C	D	O	A	I	R	C	D		
G1	1	-	-	-	-	-	-	-	-	-	-	-	-	0.33	Non irritating
	3	-	-	-	-	-	0.15	-	-	-	-	-	0.33		
	6	-	-	-	-	-	-	-	-	-	-	-	-		
G2	1	-	-	-	-	-	-	-	-	-	-	-	-	0.33	Non irritating
	3	-	-	-	-	-	0.15	-	-	-	-	-	0.33		
	6	-	-	-	-	-	-	-	-	-	-	-	-		
G3	1	-	-	-	-	-	-	-	-	-	-	-	-	0.33	Non irritating
	3	-	-	-	-	-	0.15	-	-	-	-	-	0.33		
	6	-	-	-	-	-	-	-	-	-	-	-	-		

(O) Opacity; (A) Area involved; (I) Values for congestion and hemorrhage; (R) Redness; (C)Chemosis; (D) Discharge; (-) No score observed

Corneal residence Evaluation

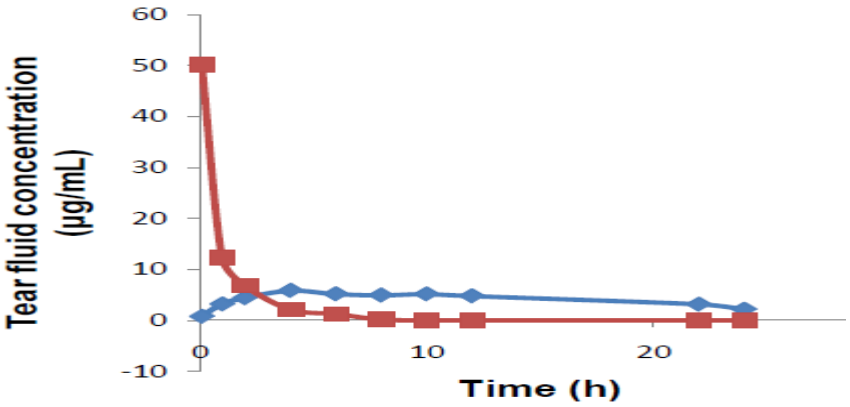


Table: 14 Stability studies Report for F8 formulation

S. no	Observation	Before Stability testing	During Study		
			30Days	60 days	90 Days
1	Clarity	Clear	Clear	Clear	Clear
2	Visual Appearnce	Transparent	Transparent	Transparent	Transparent
3	pH	6.8	6.8	6.8	6.79
4	Drug Content	99.1%	99.1%	99.1%	99.0%

The Detachment Stress was determined for ophthalmic gels. Results of this test indicate that the variable Carbopol 940 and HPMC K15M both are having effect on bioadhesive strength. It shows that bioadhesive force was increased with the increasing concentration of the Carbopol 940 and HPMC K15M.

Drug content: Good uniformity in the **drug content** among the batches was observed for all the formulations. Drug content for Gatifloxacin was found in the range of 96.07% to 101.13%. for Dexamethasone it was found in the range of 90.17% to 99.23%.

The drug content analysis of the prepared formulations have shown that the process employed to prepare films in this study was capable of giving films with uniform

drug content and minimum batch variability

Rheological Study: 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.

Sterility Study: There was no a earance of turbidity and hence no e idence of bacterial growth when optimized formulation was incubated for days at 30^0 -35 ^{0} c in case of fluid thioglycolate mediumand at 20- 25 ^{0} C in case of soyabean-casein digest medium. The preparations examined, therefore, passed the sterility test.

***In-vivo* Study**

Eye irritation test

Ocular Safety score for insitu gel in Rabbit eyes: No irritation was found due to insitu gel

Corneal residence Evaluation: There was a significant improvement in precorneal residence of drug after application of the formulated insitu gel as compared to eye drops. In case of insitu gel, the levels of drug concentration in tear fluid were maintained for 10 hr while for eye drops concentration was very less after 6 hr. The increase in corneal residence may be attributed to the controlled release of drug from the insitu gel as proved by *in vivo*-*in vitro* release studies

Stability Studies: From stability study it reveals that optimized F8 formulation showed no change in physical appearance, but both formulations showed very less decrease in drug content and in vitro release. This may because of slight degradation of drugs at elevated temperature, but these changes will not occur if formulations store at room temperature. From study it reveals that tested formulation were satisfactorily stable.

CONCLUSION

The Present Study was mainly focused on investigating influence of Carbopol 940 and HPMC K15 M polymers and their concentration on ocular delivery using factorial design statistically. Insitu gel of all batches had desired ocular physicochemical properties. Both polymers amount and their ratio had significant influence on dependent variables studied. The ratio of hydrophilic and hydrophobic polyme affected the gelling capcity, bioadhesive strength, rate of drug release and consequently the permeation of the drugs.

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