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Research Article

Formulation and *In-vivo* Evaluation of ocular insitu gel of Gatifloxacin by temperature triggered method

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ABSTRACT

The main aim of the present investigation was formulation and evaluation of ocular insitu gel of Gatifloxacin by temperature triggered method for bacterial conjunctivitis. The preformulation studies for the drugs included API characterization, solubility, melting point, determination of λ max, standard calibration curve drug and excipient compatibility study was carried out. The ocular insitu gel of Gatifloxacin was prepared by cold method. Total nine formulation (F1-F9) of ocular *insitu* gel was prepared with different concentration of Pluronic F127(15,18,20%) and HPMC E50 LV(0.5,0.75,1.0%). They were evaluated for clarity, appearance pH, Drug content uniformity, Gelling Capacity, Gel temperature determination, viscosity studies, Sterility studies, isotonicity studies, *In-vitro* diffusion studies, and *In-vivo* studies. The formulation (F5) was selected as the best formulation because of its optimum viscosity and good gelling capacity. Drug content was found to be 98.85± 1.03% . it gives the maximum *in-vitro* drug release 90.80% for 12 hours. The eye-irritation study on albino rabbits revealed no signs of eye irritation, swelling , edema and cloudiness. The stability studies were performed on F5 formulation at two different temperature of 25± 2ºC /60%□5%RH and 4± 2ºC/40%RH for the period of 3 month shows satisfactory results. The result confirmed ocular *insitu* gel as potential candidate for bacterial conjunctivitis.

Keyword: Ocular *in-situ* gel, Bacterial conjunctivitis, Pluronic F127, HPMCE50LV,Temperature triggered method.

INTRODUCTION

In-situ gelling drug delivery system providing controlled and sustained manner of drug release, for archiving constant drug plasma concentration. It can be very easily applied to the site of drug absorption in the form of liquid, at the site of drug absorption it will convert into gel form and providing increased residence of drug release at the site of drug administration. It provides ease drug administration, reduced frequency of drug administration and reducing toxicity of drug. Synthetic as well as natural polymers are used for the preparation of insitu gel. *Insitu* gel formation occurs due to the one or combination of stimuli like pH change, Temperature modulation, presences of ions, crosslinking of enzymes, and photo-polymerization.

Broad spectrum antibiotic are used to treat bacterial conjunctivitis. A broad spectrum antibacterial agent called Gatifloxacin is used in treatment of bacterial conjunctivitis. It isavailable as 0.3 and 0.5% solution and is usually administrated two to three daily. Ocular residence time is shortened as a result of rapid removal of drug solution from the surface of cornea by lacrimation this leads to poor bioavailability, and precorneal elimination. Utilizing in- situ gelling ophthalmic medication delivery methods can help to overcome these drawback of thesolution. This system can be prepared as a liquid dosage form for ease administration in to the corneal surface of eye, later this solution converted into gel form because of physiological condition. This helps to increasing the precorneal residence time, reduce the drug loss from the corneal surface and improve the bioavailability of Gatifloxacin.

The goal of this research project was to develop, *in-vitro* and *in-vivo* evaluation of ocular *In situ* gel using various polymers such as HPMC E50 LV and Pluronic F127 by temperature triggered method to overcome the drawbacks of Gatifloxacin eye drop that is currently available in the market , that improving of ocular availability of Gatifloxacin , avoidance of first pass metabolism, improving physiochemical and pharmacological response, Pluronic F127 and the viscosifying agent HPMC E50LV are used in combination to give a sustained and controlled release of the ocular *insitu* gel delivery system.

MATERIAL AND METHODS

Material

Gatifloxacin was obtained from yarrow chem

product, Mumbai. Pluronic F127 was obtained from sigma Aldrich Chemicals Pvt Ltd. HPMC E50 LV, Sodium chloride , Benzalkonium chloride, Calcium chloride dihydrate and Sodium bicarbonate was obtained from S.D Fine Chemicals Pvt Ltd Mumbai, India. All chemicals and solvents used were of analytical grade.

Methodology Preformulation studies

Solubility: The solubility of the selected drug was determined in Dimethyl formamide, DMSO, Distilled water, Ethanol, Acetic acid, Stimulated tear fluid.

Melting point: Fine powder of Gatifloxacin was filled in a glass tube (previously sealed at one end) and attached to a thermometer with rubber band, was immersed in the Thiel's tube containing liquid paraffin. Heating was commenced. The melting temperature was determined.

Fourier Transform Infrared Radiation (FTIR): FT-IR studies were performed with drug alone and with other excipient to find compatibility between drug, Pluronic F127, and HPMC E50 LV used to formulate Insitu gel of Gatifloxacin. A Tensor 27 Instrument was used for studies using the KBr pellet method.

Preparation method of Ocular *insitu* gel

Temperature triggered Pluronic F127and HPMC E50 LV based system of ocular *in situ* gel Gatifloxacin ocular *insitu* gel was prepared with cold method. Drug Gatifloxacin and Nacl an isotonicity adjusting agent were dissolved in distilled water and kept the resulting solution in refrigerator for cooling. After cooling 15, 18, 20 % of Pluronic F127 was weighed and dissolved in the distilled water then kept the solution of different concentration of Pluronic F127 in refrigerator at 4□C. In order to complete dissolution of the viscosity enhancing agent, the necessary quantity of HPMC E50 LV was dissolved in hot water at 80-90ºC with constant stirring. The prepared solution of various concentrations HPMC E50 LV and Pluronic F127 were then added into the solution of drug and Isotonicity adjusting agent. The resulting solutions of polymers were kept for cooling in refrigerator. Benzalkonium chloride (0.02%) was added as a preservative for avoiding the microbial growth. The polymeric solution of HPMC and PF127 with different concentration grade were prepared and evaluated for appearance clarity, and gelling capacity, gelation temperature at physiological condition.

EVALUATION OF OCULAR *INSITU* GEL OF GATIFLOXACIN

Determination of Clarity and pH

The appearance and clarity of the prepared formulation was determined by visual observation. A digital pH meter was used to measure the pH of the prepared ocular *insitu* gel of Gatifloxacin.

Gelation temperature determination

Gelation temperature were determined for all prepared formulation of Gatifloxacin by taking 2-3 drops of refrigerator sample into the test tube containing stimulated tear fluid. Then these test tubes were placed on the water bath. The solutions were heated at constant rate of 1□C every 1 min. Note down the temperature at which solution converted into gel form. Measurement was carried out in triplicate for each formulation.

Drug Content

Drug content estimation was help to determine the dose uniformity of drug in each formulation that is degree of uniformity. Weighed amount of gel of prepared formulation of Gatifloxacin wasdiluted with stimulated tear fluid (7.4). Then the solution was filtered. Collect the filtered drug solution. The resulting sample's absorbance was measured at 286 nm.

Gelling Capacity

All prepared formulations gelation capacity was determined by visual inspection and then it was graded in three categories on the basis of a integrity, stiffness and time period for which the gel retained its rigidity. 2 drops of the prepared formulation of Gatifloxacin in a beaker containing stimulated tear fluid (7.4) and observing the time at which formulation converting in the form of gelation and also time taken for the prepared gel to redissolve completely.

Rheological study

Brookfield viscometer was used for viscosity study using spindle LV3. Approximately 30 ml of prepared sample transferred into 50 ml beaker

which was set up to spindle groove was dipped on it at different rpm and result were given in table 2.

In-vitro Diffusion studies

Franz diffusion cells were used to conduct *In-vitro* diffusion studies, Ocular *insitu* gel was kepton the membrane mounted on donor compartment and stimulated tear fluid pH 7.4 was filled inside the receptor compartment. The receptor compartment was constantly stirred at 100 rpm while the temperature was kept at 37±0.5ºC. At different time interval, 1 ml of sample was withdrawn from receptor compartment and replaced with freshly prepared stimulated tear fluid.After that, samples were spectrophotometrically examined at 286nm.

Drug release kinetic study

The kinetics of release of the drug in different formulation was studied by various kinetic models such as kinetics of release of the drug in different formulation was studied by various kinetic models such as zero order, first order, Higuchi model and Korsmeyer-peppas model. The regression coefficient (r^2) value obtained from the graph plotted by taking Zero-order as % drug release Vs time, First order as Log% drug retained Vs time, Higuchi as % drug release Vs time, Korsmeyer-Peppas as log % drug release Vs log time gives use an idea to which the drug release follows.

Isotonicity study

Maintenance of isotonicity is very important characteristics of ophthalmic formulation. It helps to prevent the eye irritation as well as tissue damage of eye. Selected formulation F5 was subjected to isotonicity study. 1 drop of selected best formulation was mixed with blood and observed with the help of microscope at 45x magnification. The blood cells shape was compared with the marketed ophthalmic formulation containing Gatifloxacin.

Test for sterility

Ophthalmic preparation should be sterile and checked for the presence of bacteria or fungi beforeit is used .Sterility studies was performed for bacteria's like aerobic and anaerobic and fungi by using fluid thioglycolate medium(30-35□C) and soya bean casein medium (20-25□C).

Test for aerobic bacteria

Thioglycolate media was transferred into the three test tube and labeled as, test , positive control and negative control. Approximately 2 ml of the prepared formulation was transferred into the test tube labeled as test. The test tube labeled as positive controlled was inoculated with aerobic microorganism. Then these 3 test tubes are incubated at 30-35ºC for 7 days.

Test for anaerobic bacteria

Approximately 2 ml of the ophthalmic preparation was transferred into the test tube labeled as test. Approximately 2 ml of the ophthalmic preparation was transferred into the test tube labeledas test. The test tube labeled as positive controlled was inoculated with anaerobic microorganism. Then these 3 test tubes are incubated at 30-35□C for 7 days.

Test for fungi

Soya bean-casein digest medium was transferred into three test tubes and labeled as positive control, negative control, and test. Approximately 2 ml of prepared formulation was added to the test tube labeled as test. The test tube labeled as positive controlled was inoculated with fungi. Then these 3 test tubes are incubate at 20-25□C for 7 days.

In-vivo eye irritation study

White Albino rabbits of either sex (2.5 to 3 kg) was used for the eye irritation study. Selected best formulation of ocular *insitu* gel of Gatifloxacin was administered in the lower sac of conjunctiva of one eye of each rabbit. Whereas, the drug administered eye was considered as control and another eye is considered as test for preventing the loss of administered material the lower and upper eyelids of rabbit eye was closed for sometimes after the application of drug. Theobservation was done at 1, 24, 48 hours after the administration of drug. Changes occurs in eye was observed for swelling of eye, redness of eye, watering and cloudiness.

Stability studies

The stability studies were done for selected best

formulation at two different temperature i.e. refrigerated temperature (4±2ºC/40%RH) and room temperature (25±2º C/60%RH) for 3 month.

RESULT AND DISCUSSION

Gatifloxacin was very soluble in stimulated tear fluid, slightly soluble in ethanol and sparingly soluble in water. The melting point of Gatifloxacin was determined by capillary tube method. The melting point of Gatifloxacin was found to be 184ºC.

By using FT-IR Drug -excipient compatibility studies were carried out. FT-IR Spectra of pure drug showed principal absorption peaks at 3560.50 cm^{-1} (CH Stretching), 1621.40 cm^{-1} (C=O Stretching),3422.88 cm^{-1} (NH Bending) 3620.26 cm^{-1} (OH Stretching), 1290 cm^{-1} (C-O-C Stretching) The result indicates that no interaction between the drug and excipients. The peak of the drug in the spectra was also observed in the spectra of drug with excipients.

The formulation of Gatifloxacin ocular *insitu* gel F1-F9 was found to be clear, transparent andpH range was found to be with in the limit of 6.75-7.03. Gelling capacity for the formulation F1-F9 was carried out. Viscosity and gelling capacity are the two main essential requirement of gelling system. All the prepared formulation should have an optimum viscosity for easy administration into eye as a liquid which undergo sol-gel transition. Except F1 and F2 all the formulation gelled immediately and it extended for only few hours. Remaining formulation F3-F9 shows immediate gelation and it remains for the extended period of time so the optimum gelation was observed when the temperature increased to body temperature due to the optimum concentration of polymers Pluronic F127 and HPMC E50 LV.

The gelation temperature of the prepared in situ gel of the formulation was found to be in thane range between 28.7±0.27 - 35.9±0.41. The drug content of all the formulation was found in the range of 97.49±1.25 -98.85±1.03 It indicating the greater uniformity of the dosage in the formulation. Drug content of formulation F5 was found to be higher than other formulation.

All the prepared formulation of ocular insitu gel of Gatifloxacin exhibited pseudo-plastic rheology in solution form i.e.an increase angular velocity with decrease in the viscosity. As viscosity increase with decrease shear rate. This shear thinning behavior is responsible for uniform distribution of drug on corneal surface of eye. High viscosity at low shear rate increases contact time of insitu gel on corneal surface. Among all F5 gave good viscosity range.

The *in-vitro* diffusion studies of Gatifloxacin ocular *insitu* gels containing different concentrations of Pluronic F127, HPMC E50 LV were carried out for 12 hours. The percentage cumulative drug release of F1-F9 was found to be in the range of 72.95% - 90.80%. F5 showed maximum drug diffusion of 90.80% and was selected as the best formulation.

The selected best formulation was in isotonic nature because the blood cells were observed without change in their shape. It is safe for ocular use. The selected best formulation F5 of ocular *insitu* gel of Gatifloxacin showed there was no appearance of turbidity in the test tube hence; there was no presence of microbial growth. Thus, indicates the selected best formulation F5 pass the sterility test and retained its antimicrobial efficacy.

After the all section for 1^{st} ,24^{th} and 48^{th} hour rabbit eye observations, the score of rabbit eye were very less compared with the maximum total scores and the result of eye irritation study shows that there was no irritation to the ocular tissues by selected best formulation F5 of Gatifloxacin and there was no damage of ocular tissues and abnormal clinical sign hence, the selected best formulation F5 was safe for ocular use.

Stability studies was carried out for the selected best formulation at two different temperature i.e.40±2ºC/40%RH and 25±2ºC /60±5%RH for three month. Formulation was periodically evaluated for visual appearance, clarity, pH, Gelation temperature, Gelling capacity, Drug content and in-vitro drug release. The result showed that there is no much significant differencein the result.

Ingredient concentration (% W/V)									
CODE (gm)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Gatifloxacin	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Pluronic F127	15	15	15	18	18	18	20	20	20
HPMC E50 LV	0.5	0.75	1.0	0.5	0.75	1.0	0.5	0.75	1.0
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (ml)	100	100	100	100	100	100	100	100	100

Table 1: Formulation of ocular *insitu* gel of Gatifloxacin by temperature triggered method

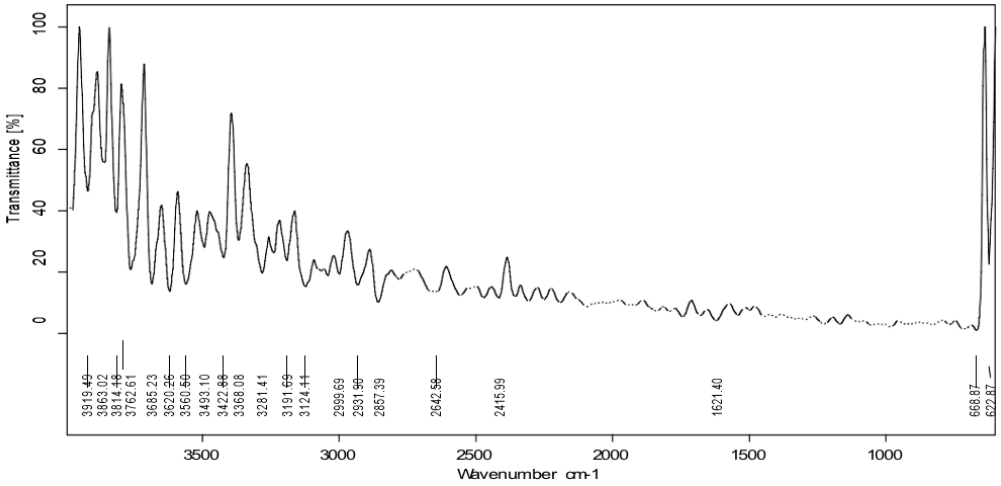


Fig 1: FT-IR Spectra of pure drug Gatifloxacin

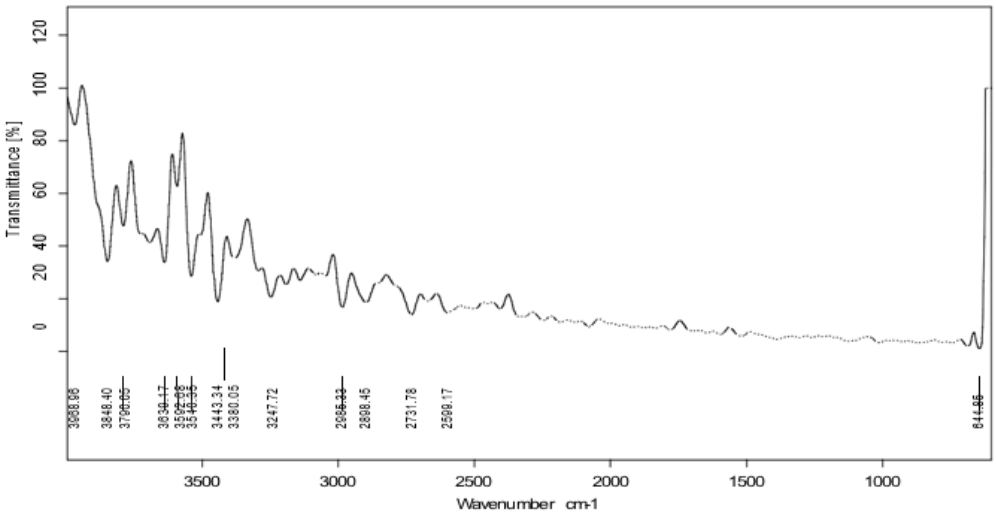


Fig 2: FT-IR Spectra of Gatifloxacin +Pluronic F127+HPMC E50LV

Formulation Code	Appearance	Clarity	pH	Gelling capacity	Gelation Temperature	Drug Content
F1	Transparent	Clear	6.84	++	35.9±0.41	96.92±1.78
F2	Transparent	Clear	6.81	++	35.7±0.23	97.76±1.14
F3	Transparent	Clear	7.02	+++	34.5±0.71	98.13 ± 1.13
F4	Transparent	Clear	6.92	+++	32.8±0.28	98.67±1.11
F5	Transparent	Clear	7.01	+++	30.3±0.24	98.85±1.03
F6	Transparent	Clear	6.75	+++	31.1±0.34	97.88±1.01
F7	Transparent	Clear	6.82	+++	30.4±0.53	98.57±1.23
F8	Transparent	Clear	7.03	+++	29.9±0.19	98.46±1.12
F9	Transparent	Clear	6.98	+++	28.7±0.27	97.49±1.25

Table 2: Physiochemical Evaluation of Ocular insitu gel

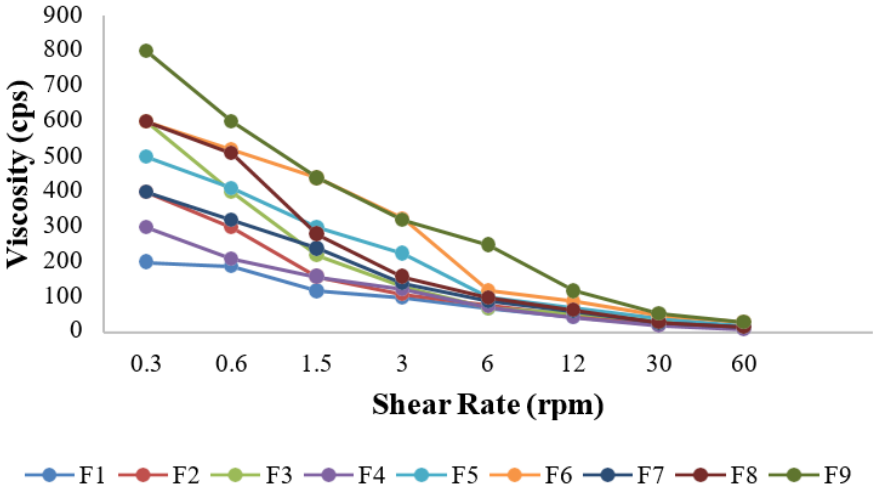


Fig 3: Viscosity of formulation F1- F9 Before gelation

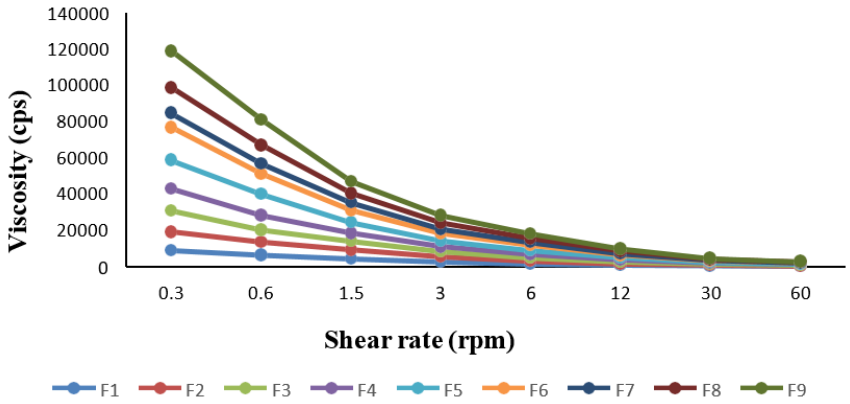


Fig 4: Viscosity of formulation F1-F9 After gelation

Time (hrs)	% CDR (F1-F9)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	13.81	12.43	11.23	12.43	13.35	11.73	11.77	12.60	12.48
2	18.22	15.37	13.35	16.96	15.22	15.21	15.32	15.32	14.56
3	23.87	20.76	16.96	22.89	20.28	19.72	24.08	20.11	20.69
4	32.53	29.41	22.63	29.41	26.83	25.98	27.58	25.28	24.37
5	39.25	35.37	28.72	37.58	35.60	36.34	31.40	32.89	29.09
6	41.78	41.86	35.19	43.07	48.73	41.34	37.18	39.59	35.21
7	46.97	43.86	40.21	56.97	55.87	48.19	42.56	45.14	40.39
8	51.27	51.73	48.35	62.09	64.94	56.23	49.123	51.37	45.67
9	62.86	58.71	57.15	70.21	69.69	62.88	56.22	57.01	51.85
10	67.97	63.33	62.09	77.59	76.43	69.34	62.39	64.30	56.36
11	79.53	69.23	68.86	82.54	82.65	76.43	69.26	70.13	65.06
12	83.58	79.53	73.15	86.25	90.80	87.59	76.88	75.66	72.95

Table 3: Drug release profile of Gatifloxacin ocular insitu gel(F1-F9)

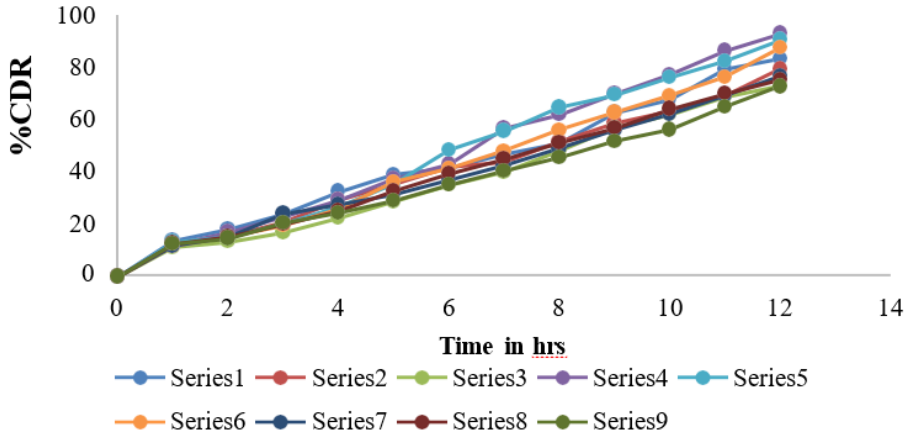


Fig 5: *In vitro* Drug Release of formulations (F1-F9) Gatifloxacin ocular *insitu* gel

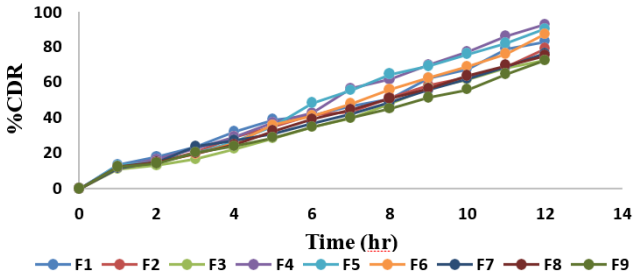


Fig 6: Zero order kinetic release (F1-F9)

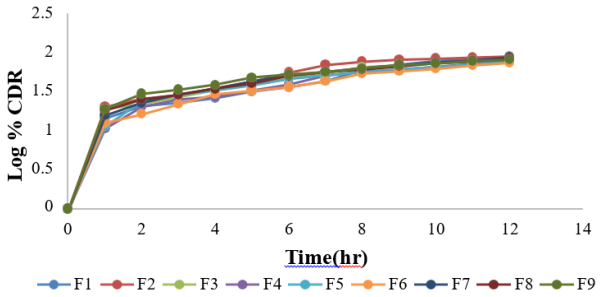


Fig 7: First order kinetic release (F1-F9)

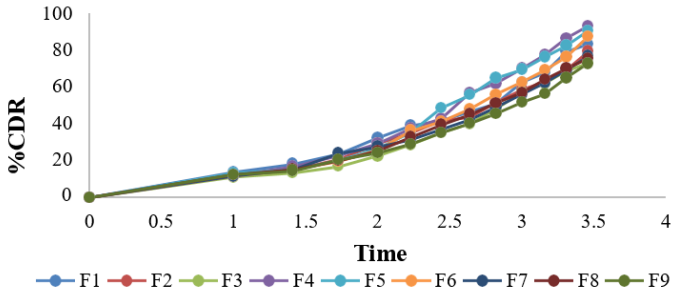


Fig 8: Higuchi plot for F1-F9 Formulation

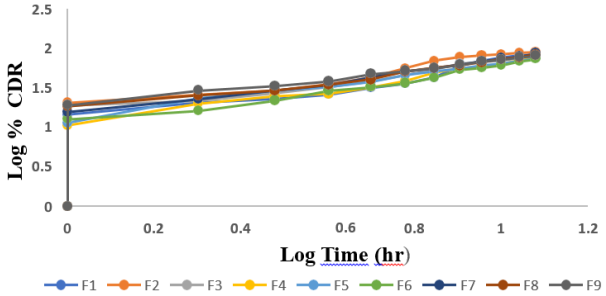


Fig 9: Korsemeyer Peppas Plot of formulation F1-F9

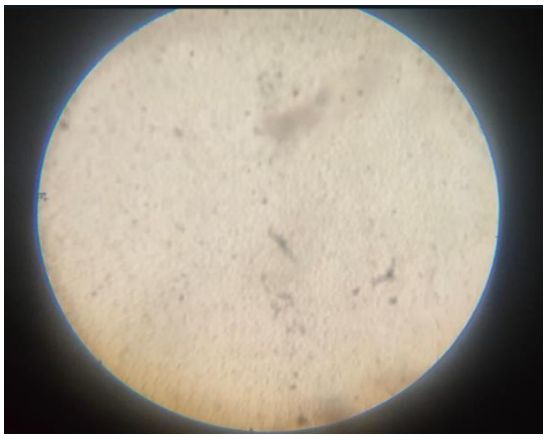


Fig 9: Blood cells with Gatifloxacin

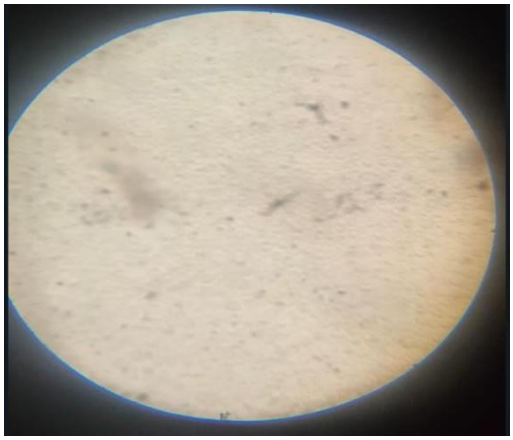
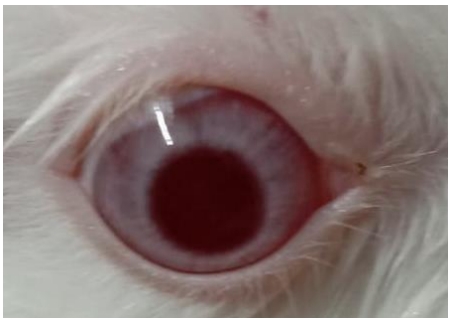
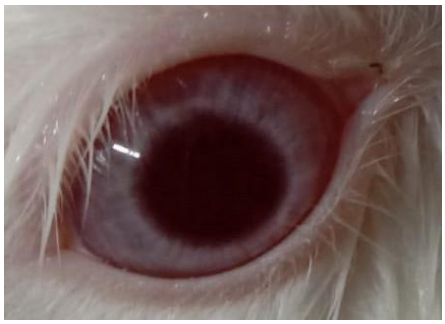
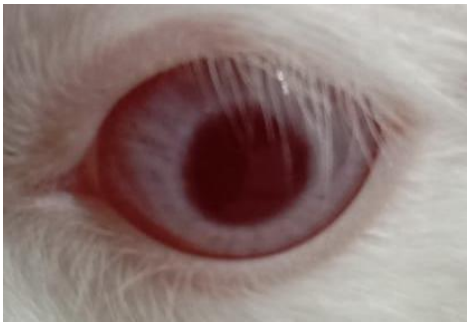


Fig 10: Blood cells with formulation F5 Sterility test

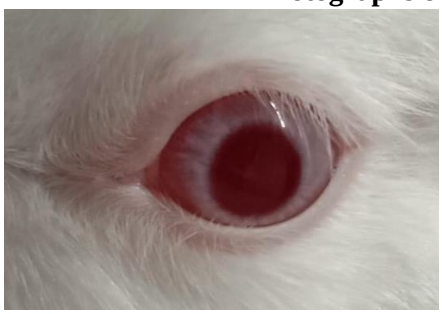
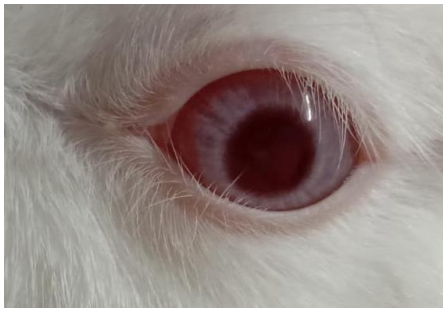




Photographs of rabbits eye after first hour

Photographs of rabbits eye after 24th hour

Photographs of rabbits eye after 48th hour

Section	Tissues	Total scores	Total Maximum Score
Section-I (1st, 24th 48th h)	Cornea	**05**	**80**
Section-II (1st, 24th 48th h)	Iris	**05**	**10**
Section-II (1st, 24th 48th h)	Conjunctivae	**06**	**20**

Table 4: Ocular irritation scores obtained for the selected formulation

CONCLUSION

Ocular *insitu* gel of Gatifloxacin for the treatment of bacterial conjunctivitis was successfully formulated with temperature triggered method with the help of various concentrations of polymers Pluronic F127 and HPMC which provides sustained release of drug. In this study, total nine Gatifloxacin ocular insitu gel formulation was prepared by using cold method. On the basis of different parameters such as drug content 98.85±1.03%, *in-vitro* diffusion of 90.80%, gelation temperature, gelling capacity, viscosity F5 was selected as best formulation and stability studies of best formulation were shows there was no significant difference in the physiochemical parameter. The formulation promises to reduce the frequency of drug administration, thus improving patient compliance. This formulation is an alternate to conventional eye drops to improve the bioavailability and it provide prolonged residence time.

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