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Formulation and Assessment of pH Triggered *In Situ* Ocular Gel using Selected Fluoroquinolone Antibiotic

***Divya Santhosh S, Dr Mathan S, Nihala Nazeer, Prof (Dr). Shaiju S Dharan**
Ezhuthachan College of Pharmaceutical Sciences, Marayamuttom, Neyyattinkara, Thiruvananthapuram, 695124

Abstract

In situ gelation is a process of gel formation at the site of application, in which a drug product formulation exists as a liquid has been transformed into gel upon contact with body fluid or at body temperature. The conventional ocular drug delivery systems show drawbacks such as increased precorneal elimination, high variability in efficiency and blurred vision. *In situ* forming polymeric formulations were developed to overcome the conventional drug therapy drawbacks. The present study deals with the formulation and evaluation of 6 (F1 to F6) formulations of pH triggered in situ ocular gel of levofloxacin using different polymer concentrations. The ocular gel was prepared by using simple dissolution method and the final formulation was sterilized by using autoclave. The prepared formulations were evaluated for visual appearance and clarity, pH, percentage drug content, *in vitro* gelling capacity, *in vitro* permeation studies, sterility test, antimicrobial efficiency test. From the results it was observed that F4 formulation showed better results in most of the evaluations. The data obtained from in vitro release study were fitted to Higuchi model and it followed first order kinetics. The present study indicates that pH triggered *in situ* ocular gel of levofloxacin could be successfully prepared in a cost effect manner and had better drug release.

Key words: Carbopol 934; HPMC K15 M; Levofloxacin; pH triggered

INTRODUCTION

In situ polymeric formulations are in sol form before administration into the body, but once administered it undergoes gelation *in situ* to form a gel. The formation of gels depends upon many factors like temperature modulation, pH change, presence of ions and ultra violet radiation etc. Mainly *in situ* gels are administered by ocular, oral, rectal, vaginal and intraperitoneal routes [1]. The *in situ* ocular gels after administered into the eye it changes from solution to the gel form and the drug released from the gel into the eye in a controlled manner so compared with other conventional dosage forms it reduces the frequency of dosing there by reduces side effects and enhance therapeutic performance of drug and they may improve the retention time of the formulation, accuracy and ease of administration [2].

The different approaches of *in situ* gel drug delivery systems are (1) physiological stimuli (e.g. temperature and pH); (2) physical changes in biomaterials (e.g. solvent exchange and swelling); (3) chemical reactions (e.g. enzymatic, chemical and photo initiated polymerization).

Temperature sensitive hydrogels are the most commonly studied class of environment sensitive polymer system in drug delivery. In this system, gelling of the solution is triggered by change in temperature. These hydrogels are liquid at room temperature (20 – 25°C) and undergo gelation when in contact with body fluids (35 – 37°C). The temperature sensitive hydrogels are categorized into three they are negatively thermo-sensitive, positively thermo-sensitive and thermally reversible gels. Negative thermo-sensitive hydrogels have a lower critical solution temperature (LCST) and contact upon heating above the LCST. One of the most extensively investigated polymers that exhibit useful LCST transition is poly (N-isopropyl acrylamide).

A positive thermo-sensitive hydrogels has an upper critical solution temperature (UCST) such hydrogel contacts upon cooling below the UCST. Polymer networks of poly (acrylamide-co- butyl methacrylate) have positive temperature dependence of swelling. Novel protein polymers called as prolastins, which undergo an irreversible sol gel transition when injected as a solution into the body, the material forms a firm, stable gel within minutes [3-6].

The pH sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groups are known as polyelectrolytes. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups but decreases if polymer contains weakly basic (cationic) groups. The pH responsive polymers are solution at pH 4 and gel at pH 7.4 [7].

In situ formations based on physical mechanisms are of two swelling and diffusion. In swelling the polymer absorbs water from surrounding environment and expands to form gel. One such substance is myverol 18-99 (glycerol mono-oleate) which swells in water to from a crystalline phase structure. In diffusion method the polymer forms gel by the diffusion of solvent from polymer solution into surrounding tissue and results in precipitation or solidification of polymer matrix [8-9].

Chemical reactions that results *in situ* gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes and photo initiated processes. In ionic cross linking polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion sensitive forms. While k-carrageenan forms elastic mainly in the presence of small amount of k^+, i-carrageenan forms elastic mainly in the presence of Ca^{2+}, Mg^{2+}, k^+ and Na^+ ions[10]. In enzymatic cross linking the gelation catalyzed by natural enzymes, this type has some advantages over other chemical reactions for e.g., an enzymatic process operates efficiently under physiological conditions without need for potential

harmful chemicals such as monomers and initiators. Photo-polymerization is commonly used for *in situ* formation of biomaterials. A solution of monomers and initiator can be injected into a tissues site and the electromagnetic radiations are used to form gel [11]. Photo polymerizable systems when introduced to the desired site via injection get photocured in situ gel with the help of fiber optic cables and then release the drug for prolonged period of time. The photo-reactions provide rapid polymerization rates at physiological temperature [12].

The barriers of ocular delivery are the major hudles for the formulation of an eye preparation. The barriers are: Drug loss from the ocular surface, over production of lacrimal fluid, blood ocular barriers. If conventional ophthalmic preparations such as eye drops or eye solutions are used, after instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye [13].

MATERIALS AND METHODS

Levofloxacin, carbopol 934, HPMC K15 M, BKC, Tween 20, citric acid, Di-sodium hydrogen phosphate, sodium hydroxide, calcium chloride, sodium chloride, sodium bi carbonate were obtained from Yarrowchem Products, Mumbai, India.

PREPARATION OF CITROPHOSPHATE BUFFER pH 6

100 ml of citrophosphate buffer pH 6 was prepared in distilled water by mixing 17.9 ml of 0.1 M citric acid solution and 30.3 ml of 0.2 M solution of dibasic sodium phosphate and diluted to 100 ml with distilled water [14].

PREPARATION OF *IN SITU* GEL

In situ ocular gel of levofloxacin were prepared by simple dissolution method (Table a). Accurate amount of HPMC K15 M was added to 75 ml of the buffer and allowed to hydrate for about 20 minutes. Carbopol 934 was then sprinkled over the solution and stirred for few minutes. The solution was then allowed to hydrate overnight. The solution was again stirred after overnight hydration for uniform dispersion of the polymer. Required amount of levofloxacin was weighed and dissolved in 20 ml of the buffer. Then, benzalkonium chloride and tween 20 were dissolved in the solution. When the drug solution and polymer solution were mixed, immediate precipitation of carbapol occurred due to the decrease in pH brought about by Carbapol. Therefore, the drug was incorporated in a sufficient quantity of 0.1M NaOH and then added to the polymer solution to get a clear solution of drug and polymer, prepared formulation were sterilized in an autoclave at 121º C for 20 min [15-16].

EVALUATION OF pH TRIGGERED *IN SITU* OCULAR GEL

Fourier-transform infrared (FTIR) spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is based on the idea of the interference of radiation between two beams to yield an interferogram. The latter is a signal produced as a function of the change of path length between the two beams. The two domains of distance and frequency are inter convertible by the mathematical method of Fourier-transformation.

Drug - excipient compatibility was determined by FTIR analysis. It is carried out by the spectral analysis of drug and drug - excipient mixture. The changes in chemical composition of drug after mixing with excipents were determined with IR spectral analysis. IR was used because mixing of the two components in the molecular level will cause change in oscillating dipoles of the molecules. If the drug and polymer interacts, then the functional groups in FTIR spectra will show the band shift and broadening compared to that of pure compounds [17 – 18].

Determination of visual appearance and clarity

The appearance and clarity were determined visually against a white and black back ground for presence of any particulate matter. This test can be performed with the help of a clarity test apparatus [19].

pH

pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. Ophthalmic formulation should have pH range in between 5 to 7.4.

The preparation to be instilled into eye should be non-irritant to the eye. To ensure that the preparation has same pH as that of lacrimal fluid, the pH of the prepared *in situ* gelling system after addition of all the ingredients was measured using calibrated digital pH meter. The digital pH meter which was calibrated using buffers of pH 4 and pH 7 before the measurement. Each recording was made in triplicates when they are in sol condition [16].

Percentage drug content

The drug content was determined by accurately measured 1 ml of formulation transferred to 100 ml volumetric flask and make up with simulated tear fluid pH 7.4. and stirred on magnetic stirrer for 1 h. The solution were filtered through a whatmann filter paper and aliquot of 5 ml of solution was withdrawn and further diluted with 25 ml of STF and the concentration was determined by UV method [19].

Viscosity and rheological studies

Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. Viscosity of the formulation increased with increase in polymer concentration. The hierarchy of shear rate was reversed and average of two readings was used to calculate viscosity.

The rheological properties of solution and gels were measured using Brookfield programmable DV-E viscometer. Spindle no. 62 was used and the angular velocity or shear rate was increased gradually from 3 to 100 rpm.

Pre - gelation viscosity studies: The pre- gelation viscosities of the formulations were evaluated to determine the viscosity of the formulations at the pH 6. Place the viscometer on a flat platform and turn on the

viscometer. Connect the spindle and the developed formulation was poured into a small beaker, the spindle immersed perpendicular in to the centre of beaker containing formulation taking care that spindle does not touch bottom of the jar. Adjust the spindle number and rotation then switch on the motor and rotated for 10 minutes then evaluate the viscosity, switch off the motor and increased the shear rate then switch on the motor and evaluate the viscosity. The shear rates were adjusted from 3 to 100 and evaluate the viscosity in each rpm.

Post – gelation viscosity studies: The post- gelation viscosities of the formulations were evaluated to determine the viscosity of the formulation after instilled in to the eye. The formulation was then poured into another beaker and the pH was raised to 7.4 by adding STF or 0.5 N NaOH. Spindle was cleaned by using water and wiped with tissue paper. The spindle immersed perpendicular in to the centre of beaker containing formulation, the spindle does not touch the bottom of the jar. Adjust the spindle number and rotation speed then switch on the motor allowed for 10 min rotation and evaluate the viscosity. [20].

***In vitro* gelling capacity**

Simulated tear fluid (STF) were prepared by using accurate quantity of sodium chloride, sodium bicarbonate and calcium chloride dehydrate (Table b). All prepared formulations were evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as *in situ* gelling systems. The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37°C and visually assessing the gel formation and noting the time for gelation and time taken for the gel formed to dissolve. The flow behavior with "+" sign indicates gelation occurred after few minutes and dissolved rapidly. The flow behavior with "++" sign indicates that immediate gelation and remained up to few hours. The flow behavior with "+++" sign indicates that immediate gelation and remains for extended period [21].

***In vitro* drug permeation studies**

The drug release from the prepared formulation was studied by bichambered donor receiver compartment model (Franz diffusion cell) using cellophane membrane soaked overnight in the receptor medium STF pH 7.4. The diffusion medium was filled in the receptor compartment and it was stirred at 50 rpm at 37 ± 1º C. One end of the diffusion tube was covered by a cellophane membrane. The 2 ml formulation was spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium present in receptor compartment. The drug samples were withdrawn at the interval of 1 h for the period of 8 h from diffusion medium and analyzed by a UV spectrophotometer at 293 nm [22].

Sterility test

It is necessary to test for sterility for all ophthalmic preparations. In this study the formulation was sterilized by using autoclave. The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. All the test glass wares and syringes are sterilized by using hot air oven. The test liquid was aseptically transferred to soyabean - casein digest medium. The inoculated media were incubated for not less than 7 days at 37 °C [23].

Antimicrobial efficacy studies

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with test organisms. The resulting zones of inhibition will be uniformily circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Agar well diffusion method was used to test the antimicrobial efficacy of prepared formulations. The materials required for this test was muller hinton agar medium, nutrient broath and test organism. *Staphylococcus aureus* (ATCC 25923) were used as the test organisms to study the antimicrobial efficiency.

Muller hinton agar medium (1 L):

The medium was prepared by dissolving 33.8 g of the commercially available muller hinton agar medium in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121˚C for 15 min. The autoclaved medium was mixed well and poured on to 100 mm petriplates (25 - 30 ml) while still molten.

Nutrient broth (1 L):

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium in 1000 ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure at 121° C for 15 min.

Petriplates containing 20 ml muller hinton agar medium were seeded with bacterial culture of *Staphylococcus aureus.* Wells of approximately 10 mm was bored using a well cutter and different concentration of sample such as 250 µg/ml, 500 µg/ml and 1000 µg/ml were added. The plates were then incubated at 37°C for 24 h. the antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Streptomycin was used as a positive control (concentration 10 mg/ml). The culture of test organisms; growth of culture adjusted to McFards Standard 0.5 % [24].

Drug Release Kinetic Study

To analyze the mechanism of the drug release kinetics of the dosage form, the data obtained were fitted to various kinetic equations of zero order, first order, higuchi model and korsemeyer-peppas model.

Table a: Composition of pH triggered *in situ* ocular gel

Ingredients	Formulation code					
	F1	F2	F3	F4	F5	F6
Levofloxacin	0.5	0.5	0.5	0.5	0.5	0.5
Carbopol 934	0.3	0.4	0.5	0.6	0.7	0.8
HPMC K15 M	0.1	0.1	0.1	0.1	0.1	0.1
BKC	0.002	0.002	0.002	0.002	0.002	0.002
Tween 20	1.0	1.0	1.0	1.0	1.0	1.0
Sodium hydroxide	qs	Qs	Qs	qs	qs	Qs
Citrophosphate buffer pH 6	100	100	100	100	100	100

All quantities are taken in grams Table a: Formulation ingredients

Table b: Composition of STF

Ingredients	Quantity
Sodium chloride	0.67 g
Sodium bicarbonate	0.2 g
Calcium chloride dehydrate	0.008 g
Water q.s to	100 ml

RESULTS AND DISCUSSIONS

Fourier-transform infrared spectroscopy

FTIR spectrum of levofloxacin showed all the peaks corresponding to the functional groups present in the structure and there were no additional peaks. The combination spectrum of drug and excipient in figure 1 and 2 also showed no change in the peak values corresponding to functional groups of the drug when combined with excipients, indicating that the drug is compatible with the excipients.

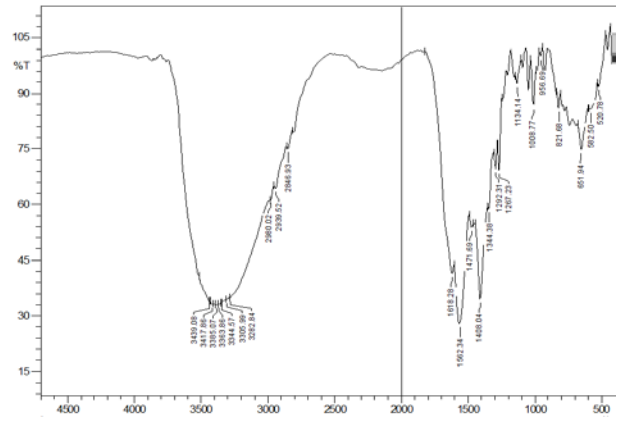

Figure 1: FTIR of levofloxacin pure drug

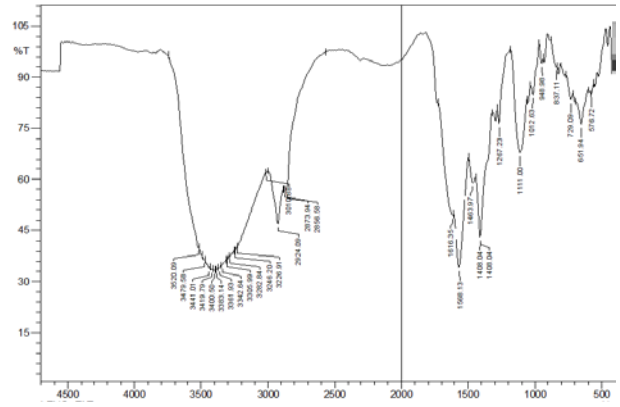

Figure 2: FTIR of levofloxacin + carbopol 934 + HPMC K15 M + NaOH + citrophosphate buffer + BKC + tween 20

FTIR analysis was carried out for pure drug and drug excipient mixtures. FTIR spectrum of drug shows the prominent peaks with respect to the functional groups. The FTIR spectrum of physical mixture of drug with excipient concluded that there is no significant interaction between the drug and excipients.

Determination of visual appearance and clarity

The visual appearance and clarity of prepared formulations were shown in Table c. The appearances of all formulations were light yellow in colour and the formulations except F5 and F6 were clear. Due to high concentration of polymers F5 and F6 were slightly translucent. The terminal sterilization by autoclaving had no effect on physicochemical properties and the clarity of the formulation.

Table c: Visual appearance and clarity

Formulations	Appearance	Clarity
F1	Light yellow colour	Clear
F2	Light yellow colour	Clear
F3	Light yellow colour	Clear
F4	Light yellow colour	Clear
F5	Light yellow colour	Slightly
F6	Light yellow colour	Slightly

Determination of pH

The pH of prepared formulations were shown in the (Table d). The pH of the formulation were found to be satisfactory and was in the range of 5.46 to 6.71 and would not cause irritation upon administration in the eyes. Terminal sterilization by autoclaving had no effect on the pH.

The acceptable pH range of ophthalmic formulations is 6.0 to 7.4. Above or below this pH range may cause eye irritations.

Table d: pH of formulations before gelation

Sl. No	Formulations	pH SD
1	F1	6.05 0.015
2	F2	5.86 0.062
3	F3	5.81 0.320
4	F4	6.71 0.037
5	F5	5.46 0.047
6	F6	5.46 0.142

* Average of 3 determinations, SD = standard deviation

Percentage Drug Content

The percentage yield of all *in situ* gel formualtions were performed and the values obtained were in the range of 95.81% to 99.76%. The data is summarized in the (Table e).

Table e: Percentage drug content

Sl.No	Formulations	Drug content (%)
1	F1	99.06 0.19
2	F2	97.20 0.16
3	F3	99.62 0.24
4	F4	99.76 0.21
5	F5	98.20 0.14
6	F6	95.81 0.11

* Average of 3 determinations, SD = standard deviation

Viscosity and rheological studies

The viscosity of all the batches were tested. The data is summarized in (Table f and g). The viscosity of all 6 formulations shows high under conditions of low shear rates. The viscosity and rheological behaviour of all the formulations before and after the addition of STF was evaluated by a Brookfield programmable DV-E viscometer by using spindle no: 62 at different shear rates. The graphical representations of viscosity v/s shear rate of in situ gel before and after addition of STF shown in (figure 3, 4, 5, 6).

The rheological behaviour of all the 6 formulations showed shear thinning, exhibiting pseudo plastic behaviour. The viscosity of formulations F1 to F6 ranged from 53 to 700 cps before addition of STF. The viscosity of the formulations F1 to F6 ranged from 600 to 4323 cps after addition of STF.

***In vitro* gelling capacity**

The gelling capacity of all batches were tested all formulations changes sol form to gel form (Figure 7). The data is summarized in (Table h). Among all the formulations, the formulations F4, F5 and F6 showed better gelling capacity. It indicates concentration of carbopol 934 increases the gelling capacity is also increases.

Table f: Pre-gelation viscosity studies of in situ ocular gel of levofloxacin

Rpm	Viscosity in cps (before gelling) SD					
	F1	F2	F3	F4	F5	F6
3.0	388 0.55	250 0.34	454 0.10	498 0.45	667 0.76	700 0.54
4.0	264 3.60	217 0.80	403 0.19	415 0.67	614 0.33	684 0.25
5.0	250 0.18	201 0.24	395 0.18	401 0.78	513 0.67	658 0.12
10	210 0.11	190 0.98	304 0.16	368 0.43	428 0.89	640 0.10
12	150 0.25	180 0.36	290 0.13	303 0.77	354 0.20	504 0.14
20	110 1.23	117 0.96	286 0.11	295 0.55	333 0.18	493 0.56
30	100 1.67	106 0.24	205 0.18	236 0.12	310 0.27	301 0.78
50	84 0.90	98 0.19	101 0.17	106 0.34	247 0.69	254 0.19
60	68 0.98	84 0.14	90 0.39	95 0.78	200 0.35	211 0.78
100	53 0.17	80 0.18	86 0.89	90 0.14	108 0.78	201 0.45

* Average of 3 determinations, SD = standard deviation

Table g: Post - gelation viscosity studies of in situ ocular gel of levofloxacin

Rpm	Viscosity in cps (after gelling)					
	F1	F2	F3	F4	F5	F6
3.0	1358 0.90	1548 0.72	1948 0.67	2439 0.56	3340 0.74	4323 0.15
4.0	13300 0459	1434 0.75	2368 0.08	2401 0.34	3688 0.46	4201 0.14
5.0	1250 0.178	1263 0.19	2034 0.04	2333 0.44	3536 0.56	3967 0.84
10	1201 0.94	1259 0.09	1987 0.18	2067 0.56	3048 0.15	3500 0.85
12	1198 0.34	1250 0.96	1834 0.23	1989 0.51	2430 0.54	3001 0.87
20	1100 0.35	1111 0.46	1733 0.45	1980 0.52	2303 0.65	2998 0.45
30	993 0.13	998 0.38	1646 0.46	1933 0.54	2128 0.65	2833 0.41
50	988 0.79	994 0.76	1427 0.87	1930 0.84	2066 0.66	259 0.86
60	965 0.46	950 0.63	1238 0.96	1924 0.45	1994 0.55	2527 0.28
100	600 0.75	880 0.85	1010 0.78	1920 0.87	1936 0.56	2500 0.96

* Average of 3 determinations, SD = standard deviation

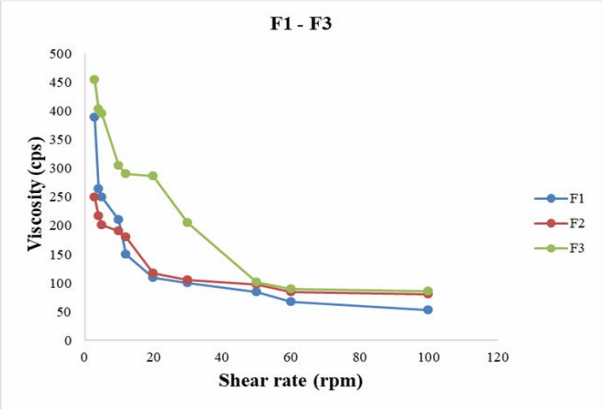

Figure 3: Pre-gelation viscosity of F1 to F3

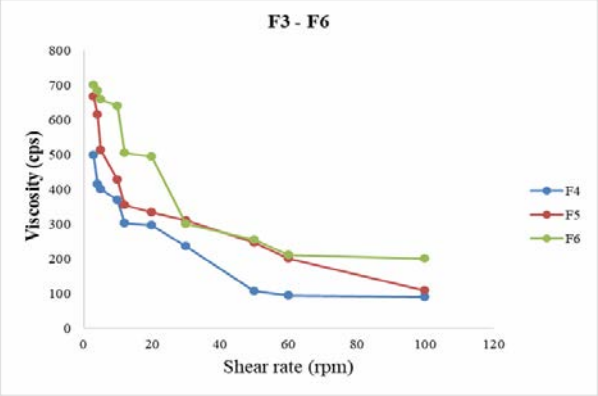

Figure 4: Pre-gelation viscosity of F3 to F6

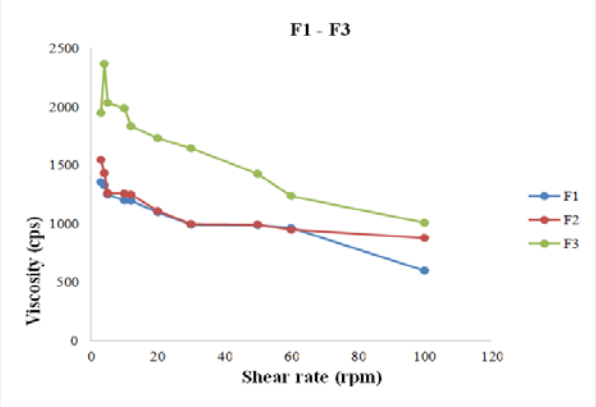

Figure 5: Post - gelation viscosity of F1 to F3

Table h: *In vitro* gelling capacity of formulations

Sl. No	Formulations	Gelling capacity
1	F1	+
2	F2	+
3	F3	++
4	F4	+++
5	F5	+++
6	F6	+++

Note: + indicates gelation occurred after few minutes and dissolved rapidly,
++ indicates immediate gelation and remained up to few hours, +++ indicates immediate gelation and remains for extended period.

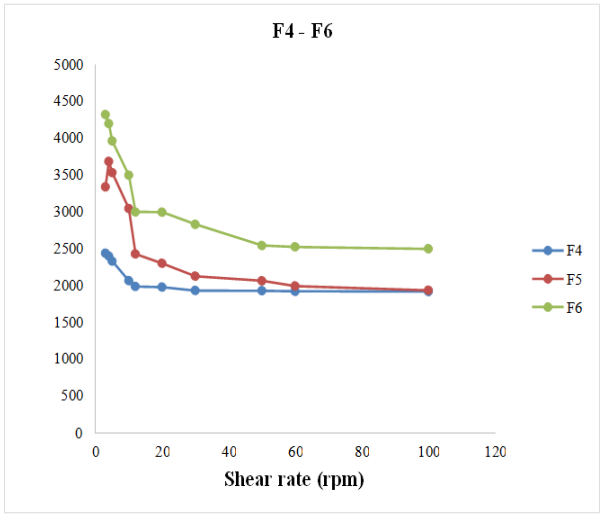

Figure 6: Post - gelation viscosity of F4 to F6

The viscosity of all 6 formulations shows high under conditions of low shear rates. The viscosity and rheological behaviour of all the formulations before and after the addition of STF was evaluated by a Brookfield programmable DV-E viscometer by using spindle no: 62 at different shear rates.
The rheological behaviour of all the 6 formulations showed shear thinning, exhibiting pseudo plastic behaviour.

Figure 7: In vitro gelling capacity

The above figure shows that the formulation at pH 5 to 6 (storage condition) in solution form. After addition of STF it converted into gel form at pH above 7.4.

***In vitro* drug permeation studies**

In Vitro permeation study of all the batches were tested by using Franz diffusion cell. The *in vitro* drug permeation of F1 varies from 19.20 to 77.324 %, F2 varies from 22.22 to 93.54 %, F3 varies from 48.86 to 97.26 %, F4 varies from 65.51 to 98.18 %, F5 varies from 44.68 to 90.31 % and F6 varies from 45.60 to 73.64 %. The F4 formulation shows best drug permeation after 8 h. The graphical representation of % CDR v/s time shown in (figure 8 and 9).

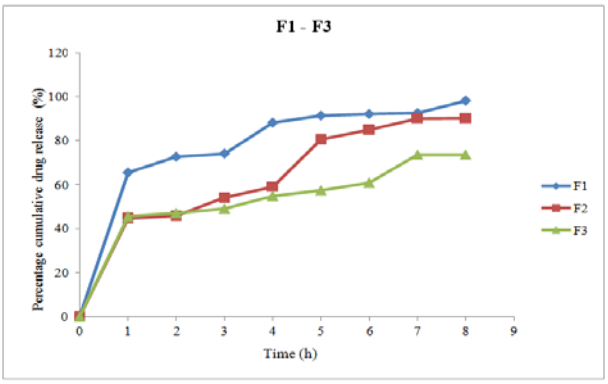

Figure 8: *In vitro* drug permeation of formulation F1 - F3

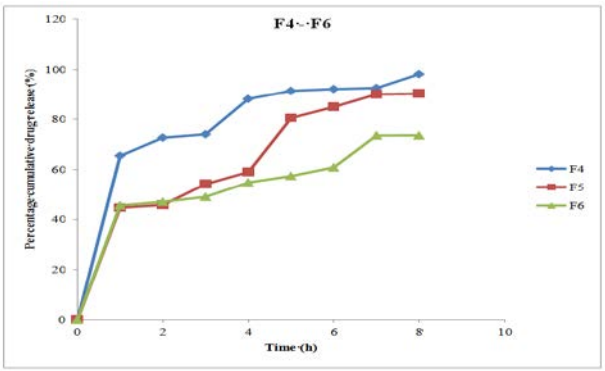

Figure 9: *In vitro* drug permeation of formulation F4 - F5

Sterility test

All the prepared *in situ* gelling system was evaluated for the sterility. After 7 days of incubation the results showed no microbial growth in all formulations (figure 10). The data is summarized in (Table i).

Table i: Sterility test

Formulation code	Incubation days						
	1	2	3	4	5	6	7
F1	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-

Table i: Sterility test data of prepared formulations Note: „-'sign indicates no growth

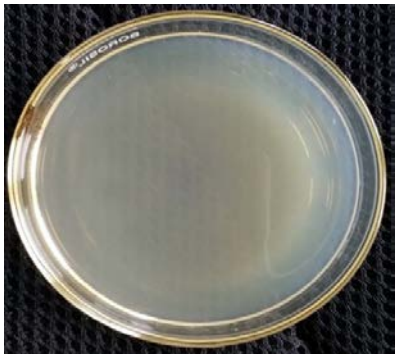

Figure 10: Sterility test

The figure shows that no microbial growth in soyabean - casein digest medium after 7 days of incubation.

Antimicrobial efficacy studies

The antimicrobial efficacy test of optimized formulation F4 was performed based on Agar well diffusion method (figure 11). The zone of inhibition of different concentration of sample was given in the (Table j). The result indicated that the selected formulation showed good antimicrobial action against the organism.

Table j: Antimicrobial efficacy studies

Concentration (µg/ml)	Zone of inhibition (mm)
Control	24
250	32
500	33
1000	35

Table j: Zone of inhibition of formulation F4 Note: Concentration of stock 10 mg/ml DMSO

Figure 11: Antimicrobial efficiency test

The figure shows the zone of inhibition of different concentration of sample of optimized formulation F4.

Kinetic modeling

The diffusion profile of optimized formulation F4 was fitted to zero order, first order, Higuchi model and Korsmeyer-Peppas model to ascertain the kinetic modeling of the drug release. The zero order rate describes the systems where the drug release rate is independent of its concentration. The R^2 value was used to evaluate the accuracy of fit. The R^2 values of each models were shown in (Table k)**,** indicate that the drug release from F4 formulation shows best fits to the first order release kinetics and the R^2 value of the first order release kinetic equations were found to be more close to unity indicating that the release from the gel is depend on the concentration of drug present in the formulation. It is also fitted to the higuchi model which gave a linear plot indicating that the mechanism of drug release was diffusion.

Table k: R^2 values of kinetic models

Formulation	Kinetic models			
F4	Zero	First	KorsmeyerPeppas	Higuchi
R^2 values	0.678	0.924	0.617	0.901

CONCLUSION

In the present study, an attempt was made to prepare pH triggered *in situ* ocular gel of levofloxacin. The *in situ* gel was prepared by using pH sensitive polymer carbopol 934 in different concentration with other excipients. FTIR studies showed that there were no marked incompatibility between the drug and polymers. The visual appearance and clarity of F1 to F4 showed good remaining 2 formulations were slightly translucent. The pH of all 6 formulations were within acceptable range of 6.0 to 7.4 and would not cause any irritation upon administration in the eyes. The viscosity and rheological behavior of all the 6 formulations were satisfied, all showed pseudo plastic behavior. The *in vitro* gelling capacity of formulations F4, F5 and F6 showed immediate gelation and remains for extended period. The *in vitro* drug permeation showed high in F4. The optimized formulation F4 showed drug permeation of 98.18 % and percentage drug content was 99.76 %. The sterility test showed no microbial growth in all 6 formulations. The optimized formulation showed good antimicrobial action against test organism. The kinetic study showed that the optimized formulation F4 follows Higuchi model followed by first order.

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