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Article

Design and evaluation of ocular hydrogel containing combination of ofloxacin and dexamethasone for the treatment of conjunctivitis

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Conjunctivitis is an inflammation of the conjunctiva, which covers the white part of the eyeball. It can be caused by allergies, bacterial or viral infection. *In situ* hydrogels are three-dimensional hydrophilic cross-linked network of polymers. *In situ* hydrogel provided better therapeutic index when compared to conventional treatment. The present work describes the formulation and evaluation of ofloxacin and dexamethasone based on the concept of pH triggered *in situ* gelation. Carbopol 934p was used as the gelling agent in combination with HPMC, as a viscosity-enhancing agent, benzalkonium chloride as preservative, sodium chloride as tonicity adjusting agent. The prepared formulations were liquid at the low pH and underwent rapid transition into viscous gel at the pH of the tear fluid. Formulations were evaluated for various rheological, *in vitro* and *in vivo* release characteristics. Infrared spectroscopy studies showed that there were no interactions between the drug and polymers. Viscosity of the prepared hydrogels lies in the optimum range and drug was released up to 85 % as the end of 13 h. The prepared *in situ* hydrogel was sterile, non-irritant to the eye. The present study indicated that it is possible to develop safe and physiologically effective *in situ* hydrogel which is patient compliant.

Keywords: Conjunctivitis. Cross link. Hydrogel. Ocular. pH sensitive.

INTRODUCTION

Designing a drug delivery system to target a particular tissue of the eye has become a major challenge for today's pharmacologist and formulation scientist due to its unique anatomy and physiology (Khan *et al.,* 2015; Mainardes *et al*., 2005). The barriers and efflux pumps in conjunction pose a significant challenge for delivery of a drug alone or in combination, especially to the posterior segment. The main purpose of ocular drug delivery is to formulate better than already existing dosage forms and to create novelty to increase the therapeutic effect (Gaudana *et al*., 2012). Conjunctivitis is an infection caused by bacteria or viruses, although lots of things like dust, sand, and pollen also can trigger it. If conjunctivitis is caused by bacteria, it may lead to crusty eyelids in the morning and also a pus formation in the eye. Conjunctivitis caused by bacteria or viruses can easily spread person to person. Bacterial conjunctivitis is caused by *staphylococci, streptococci,* or *haemophilus*. These organisms may come from another person with conjunctivitis. If conjunctivitis is not treated it may cause more serious eye problems, such as glaucoma (Drusano *et al.,* 1986). Currently eye drops are available in the market to treat conjunctivitis but they have various drawbacks like rapid elimination and short duration of therapeutic effect making a frequent dosing regimen necessary which is patient noncompliant.

Hydrogels are three-dimensional networks of polymers-, when water penetrates in to these networks it causes swelling and gives hydrogel a soft and rubbery consistency. Hydrogels are mainly of two types- preformed hydrogels and *in situ* hydrogels. *In situ* hydrogels have several advantages like prolonged drug release, reduces frequency of administration and leads to better patient

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compliance. They can be made virtually from any water-soluble polymers, encompassing a wide range of chemical compositions and wide physical properties (Nagam *et al.,* 2016). They are endowed with ability to swell in water or aqueous solvents- the highly porous can easily be tuned by controlling the density of cross links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Their porosity also permits loading of drugs in to gel matrix and subsequent drug release at a rate dependent on diffusion coefficient of the small molecule or macromolecule through the gel network (Hoare, Kohane, 2008). The benefits of *in situ* hydrogels for drug delivery are especially where a depot formulation is created from which drugs slowly elute, maintaining a high concentration of drug in the surrounding tissues over an extended period, although they can also be need for systemic delivery. *In situ* hydrogels are also generally highly biocompatible, as reflected in their successful use in the peritoneum and other sites *in vivo.* Biocompatibility is promoted by the high-water content and the physicochemical similarity of *in situ* hydrogels to the native extracellular matrix, both compositionally (particularly in the case of carbohydrate-based hydrogels) and mechanically (Shastri, Patel, 2010).

Ocular therapy would be significantly improved if the pre-corneal residence time of drugs could be increased. Many new preparations have been developed for ophthalmic use not only to prolong the contact time on the ocular surface but also to avoid drug elimination. From the point of patient acceptability, to improve bioavailability, to provide controlled release, to decrease the wastage of drug due to corneal drainage, to prevent dilution by tears, to improve corneal penetrability a study of *in situ* gels, that is instilled in-to cul de sac region will be advantageous (Zhidong *et al.,* 2006). There are four broadly defined mechanisms used for triggering the *in situ* gel formation of biomaterials: Physiological stimuli (e.g., temperature and pH), ion activated system, physical changes in biomaterials (e.g., solvent exchange and swelling), and chemical reactions (e.g., enzymatic, chemical and photo-initiated polymerization) (Kaur, Singh, Kanwar, 2000). In the present study *in situ* gel based on physiologic stimuli formation of gel is induced by pH changes. The drugs formulated in liquid solutions have several limitations, including limited bioavailability and propensity to be easily removed by tear. The low pH of the poly acrylic acid (PAA) solution would cause damage to the surface of the eye before being neutralized by the lachrymal fluid. This problem was solved by partially combining PAA with HPMC, a viscous enhancing polymer, which resulted in pH-responsive polymer mixtures that were solution at pH 4 and gel at pH 7.4 (Mitan *et al.,* 2007).

The present study was planned to prepare ocular *in situ* hydrogel containing combination of ofloxacin (OFL) and dexamethasone (DX), for the treatment of conjunctivitis using various polymers. OFL is a synthetic antibiotic of the fluoroquinolone drug class considered to be second generation fluoroquinolone and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called *DNA gyrase*, which follows the untwisting required to replicate (Nelson *et al.,* 2007; Drlica, Zhao, 1997). DX is a glucocorticoid class of steroid drug. It acts by binding with high affinity to specific cytoplasmic glucocorticoid receptors. This complex bind to DNA elements which results in a modification of transcription and protein synthesis in order to achieve inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in inflammation (Tsurufuji, Kurihara, Ojima, 1984). The combination of OFL and DX is available in the market as eye drops. Drawbacks of eye drops are rapid elimination and less contact time, results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. *In situ* hydrogels formation is more preferred for efficacy of the formulations as it remains for longer duration thereby having better activity (Panchasara *et al*., 2015).

So here, we planned to prepare ocular *in situ* hydrogel containing a combination of OFL and DX, for the treatment of conjunctivitis using different polymers. The in situ hydrogel for ocular delivery, may provide better compliance, better bioavailability, decrease the wastage of drugs due to corneal drainage, prevent dilution by tears and improve corneal permeability. Further, it may reduce the irritation.

MATERIAL AND METHODS

Material

Ofloxacin gift sample was provided by Ipca laboratories Ltd, Mumbai. Dexamethasone was procured from Matish Health Care, Indore. Carbopol 934p and HPMC were procured from Yarrow Chem. Products, Mumbai. Benzalkonium chloride was procured from Ozone International, Mumbai. Fluid thioglycolate and soyabean casein digest medium procured from HiMedia Pvt Ltd, Mumbai. All the reagents were of the analytical grade.

Methods

Preparation of *in situ* hydrogel

In situ hydrogels were formulated by using OFL, DX, benzalkonium chloride (preservative), ethylene diamine tetra acetic acid (EDTA) (chelating agent), sodium chloride (tonicity contributors) and hydroxy propyl methyl cellulose (viscolizer). Weighed quantities of OFL, DX, BZK, EDTA, NaCl were dissolved in the phosphate buffer pH 4 under aseptic condition (Table I). Then carbopol 934p was slowly added with continuous stirring with digital remi stirrer at speed of 1500-2000 rpm to minimize the formation of the lumps, and then HPMC was added with a slow stirring. Stirring was continued until a clear dispersion was formed (Singh *et al.,* 2010).

TABLE I - Formulation of *in situ* hydrogel

Formulation	OFL (mg)	DX (mg)	Carbopol (mg)	HPMC (mg)	EDTA (mg)	BZK (ml)	NaCl (mg)	pH 4 buffer (ml)
F1	150	50	0.4	0.5	0.1	0.01	0.9	50
F2	150	50	0.4	0.6	0.1	0.01	0.9	50
F3	150	50	0.4	-	0.1	0.01	0.9	50
F4	150	50	0.45	-	0.1	0.01	0.9	50
F5	150	50	0.45	0.5	0.1	0.01	0.9	50
F6	150	50	0.45	0.6	0.1	0.01	0.9	50

OFL: Ofloxacin, DX: Dexamethasone, HPMC: Hydroxy propyl methyl cellulose,

EDTA: Ethylene diamine tetra acetic acid, BZK: Benzalkonium chloride, NaCl: Sodium chloride.

Evaluation of the hydrogels

Fourier transforms infra-red spectroscopy studies

Fourier transforms infra-red spectroscopy (FT-IR) study was conducted to investigate and predict any physicochemical interaction between drug and polymers in the formulation (Makwana, Patel, Parmar, 2016).

Physical characteristics

The physical appearance of all the developed formulations was visually observed. All the developed gels were observed for its clarity. The gelling capacity was determined by placing a drop of the formulation in a vial containing 2 mL of freshly prepared STF and visually observed. The time taken for its gelling was noted (Imam *et al.,* 2018; Singh *et al.,* 2010).

Determination of pH

Initially the digital pH meter was calibrated. The pH was noted after bringing the electrodes of pH meter in contact with the surface of the formulation and allowing equilibrating for 1 min. The average of triplicates for each of the formulation was taken (Ameeduzzafar *et al.,* 2018; Balasubramaniam, Pandit, 2003).

Viscosity determination

Viscosity determinations of the prepared formulation were determined by using Brookfields viscometer LVDV II (Elscolab Netherlands B.V Tolboomweg, The Netherlands). The viscosity of the *in situ* hydrogel was measured at particular spindles (10, 30, 50, 60, and 100) (Singh *et al.,* 2010).

In vitro release study

The *in vitro* release of OFL and DX from the formulations was studied through biological egg membrane using a fabricated dissolution testing apparatus. The dissolution medium used was freshly prepared artificial tear fluid (pH 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 suitable diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 286 nm and 241.4 nm with the use of simultaneous estimation method (Hsiue *et al.,* 2002).

Release kinetics

The release kinetics was evaluated considering four different models including zero order, first order, higuchi's and korsmeyer's equation and the selection was based on the comparisons of the relevant correlation coefficients and linearity test (Shoaib *et al.,* 2006; Reddy, Mutalik, Reddy, 2003).

Test for sterility

The sterility testing of the hydrogels was performed for the aerobic, anaerobic bacteria and fungi by using alternative fluid thioglycolate medium and soyabean casein digest medium. The medium was prepared by dissolving 500 mg of peptic digest of animal tissue (such as bacteriological peptone) or its equivalent in water to make 100 mL, and the pH was adjusted to 7.1 ± 0.2. The medium was filtered or centrifuged to clarify and dispensed into flasks in 10 mL quantities and was sterilized at 121°C for 20 min. The positive control (growth promotion) and negative control (sterility) test were also carried out. Incubation was carried out in all cases and growth was observed (Soppimath *et al.,* 2002).

Antibacterial activity

Antimicrobial activity was determined by agar diffusion test employing cup plate method. The nutrient agar media was prepared and sterilized at 15 lb/sq-inch pressure for 18 minutes in an autoclave; 0.5 mL of microorganism suspension was poured into the above medium. This was done in an aseptic condition. Immediately 20 mL of the microbial agar suspension was poured into each petri plate. Standard minimum inhibitory concentration (MIC 2 µg/mL) of control and developed formulations containing ofloxacin were prepared. After solidification of the media, sterile solutions of ofloxacin (standard solutions) and the developed formulations diluted suitably with sterile distilled water (test solutions) were poured in to the cup of sterile nutrient agar petri plates. This was previously seeded with test organism. After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37°C for 24 hours. The zone of inhibition (ZOI) was measured by using antibiotic zone reader around each cup and compared with that of control. The entire operation was carried out in a laminar airflow unit. Each formulation solution was tested in triplicate (Mandal *et al*., 2012).

Ocular irritation studies

Ocular irritation study was performed on white rabbits, weighing 2-3 kg. Animals were housed in standard cage. They were fed with suitable diet and water as much as required. A dark and light cycle of 12 h was maintained. The temperature and humidity were maintained at 28 ± 2°C and 60 ± 15°C, respectively. The formulations F2 and F6 were selected for the ocular irritation study and applied once in a day for a period of 7 days. Periodically animals were observed for irritation, inflammation etc. One eye was considered as test and other as control. (Briedis, Robson 1976). The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, were followed and the present study was approved by the Institutional Animal Ethics Committee for conducting the experiment. Approval ID (SDCP/ IAEC-05/2016-17).

In vivo studies

Pseudomonas aeruginosa (*P. aeruginosa*) causes severe and rapid ocular infection and is one of the most common causes of bacterial conjunctivitis. In the study, rabbits were induced conjunctivitis by swabbing the sterile cotton which was dipped in the culture of the microorganism of *Pseudomonas aeruginosa*. The left eye was considered as a test eye (induced conjunctivitis initially followed by formulation application) and right eye was considered as a control. (Yadav, Parvez, 2008). Rabbits developed conjunctivitis symptoms 48 h after the inoculation of bacteria into eyes. The marketed product for group I and formulation F2 for group II, was placed into the eyes 24 h after the development of infection (complete development of infection), and observed for the recovery of infected eye day by day. Treatment effects were compared with those of the marketed formulation.

This study was conducted in accordance with CPCSEA guidelines, and the experimental protocol was approved by the Institutional Animal Ethics Committee (SDCP/IAEC-05/ 2016-17).

Stability Studies

Stability studies were performed as per the ICH guidelines. The *in situ* hydrogels were packed and stored at ambient humidity conditions at refrigerated temperature (2-8°C), room temperature (27°C ± 2°C), oven temperature (45°C ± 2°C) and (75 ± 5 % RH) for a period of 60 days. The optimized formulations were evaluated for changes in physical characteristics and percentage drug release (Mandal *et al*., 2012).

RESULT AND DISCUSSION

This study was undertaken to design, formulate and evaluate OFL and DX *in situ* hydrogels for the purpose of sustained release of drugs and thereby improve frequent administration and drug delivery efficiency. The prepared *in situ* hydrogels evaluated for various parameters and stability of the prepared formulations were checked as per the ICH guidelines. The results obtained were within the range.

Drug polymer interaction

The presence of any drug excipients interaction in the formulation was studied by performing FT-IR for the mixture of drug and other excipients. The FT-IR peaks of the drug: polymer mixture was compared with the main peaks of the drug in the literature to observe any changes. The important peaks of the drug OFL were observed at 1715 cm^{-1} (C=O stretching) of carboxylic acid. Other peaks were observed at 1400 cm^{-1} which is vibration associated with the protonation N_4 in the piperazinyl group, 1530 cm^{-1} which corresponds to the C=O aromatic stretching and 1055 cm^{-1} which corresponds to the C-O-C stretching of the ether group (Figure 1A). The peaks of the drug DX were observed at 1705 cm^{-1} (C=O unconjugated ketone stretch), 1655 cm^{-1} (C=O conjugated ketone stretch) and 1615 cm^{-1} (C=C conjugated stretch) (Figure 1B). The characteristic peaks of OFL and DX were approximately matched with the formulation mixture (Figure 1C). Hence, it was concluded that there was no interaction between the drug and the polymers used in the formulation of the *in situ* hydrogels.

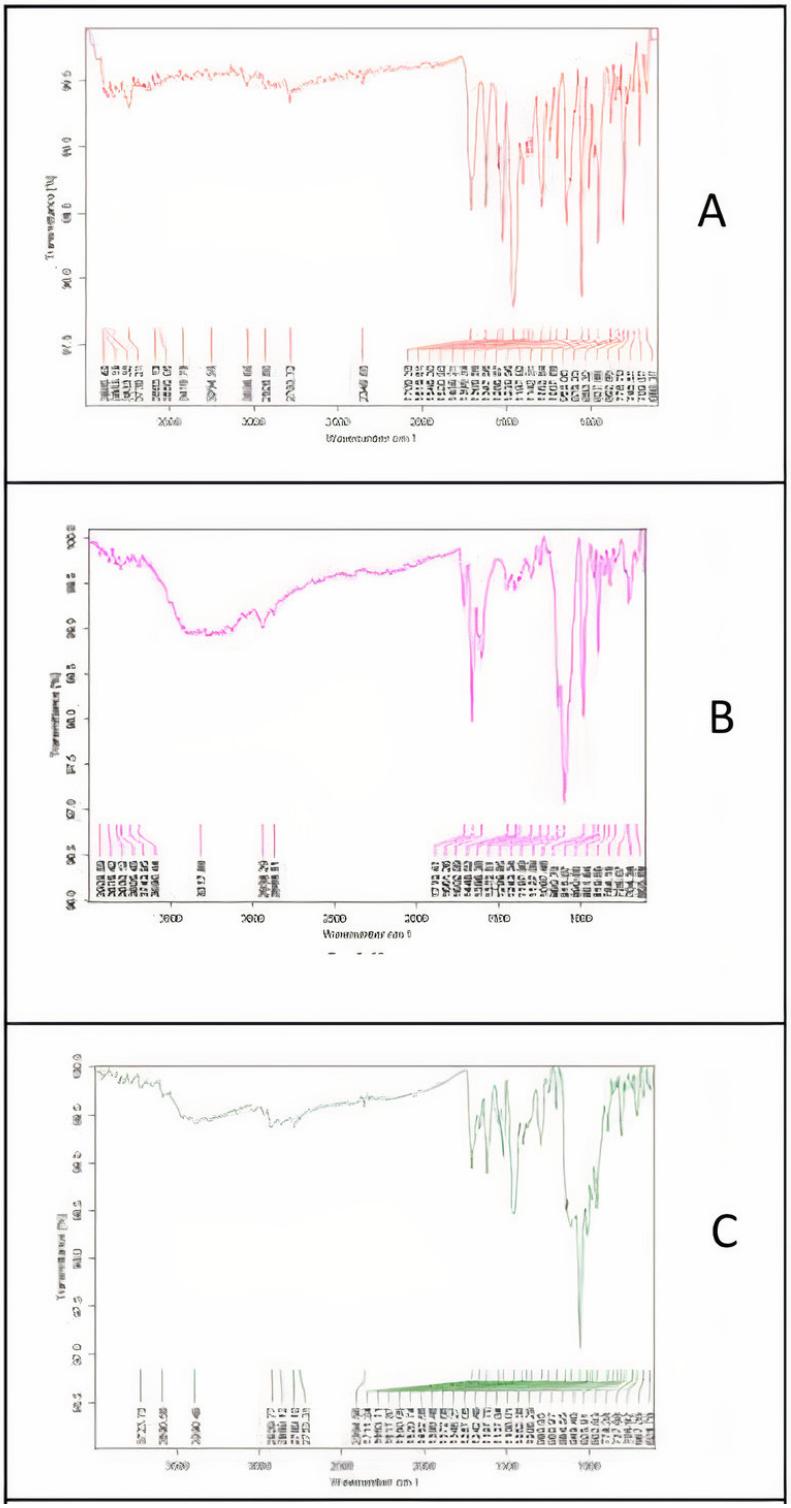

FIGURE 1 - (A) – FT-IR spectra for OFL. **B) –** FT-IR spectra for DX. **(C) –** FT-IR spectra for formulation.

Physical characteristics

Developed *in situ* hydrogel of OFL and DX was evaluated for various characteristics. The prepared formulations were white in color and all formulations were found to be clear. All the formulations were in liquid phase at pH 4 and an increase in the viscosity was observed when the pH of the formulations was raised to 7.4 which transformed into gel phase. The pH of the formulation lay between the range of 7.2-7.4. The capacity of gel was evaluated based on rate of gelation and time until the gel completely dissolved in simulated tear fluid. The following key factor was used to evaluate the gel capacity i.e., +: gels after few min and dissolves within 45 min, ++: gelation immediate, remains for few hrs, +++: gelation immediate, remains for extends period. The observed findings have been noted previously for nepafenac *in situ* hydrogel (Shelley *et al.,* 2018). Viscosity results revealed that prepared formulations were less viscous at low pH and high viscous at high pH. The viscosity lay in the range of 27-57 cps. (Dubey, Prabhu, 2014) reported that the viscosity value in the range of 15-50 cps significantly improves the contact time of the formulation on the corneal surface. The appearance, clarity, pH, gelling capacity and viscosity results obtained were within the range. The results are shown in Table II.

TABLE II - Physical characteristics of the formulations

Sample Code	pH	Clarity	Gelling capacity	Visual Appearance	Viscosity (cps)	
					pH 4 buffer	pH 7.4 buffer
F1	7.21±0.09	Clear	++	Transparent	19.6±0.3	22.5±0.2
F2	7.44±0.14	Clear	+++	Transparent	24.3±0.2	37.8±0.1
F3	7.35±0.11	Clear	+	Transparent	2.9±0.3	5.4±0.3
F4	7.29±0.12	Clear	+	Transparent	4.9±0.5	9.5±0.5
F5	7.34±0.13	Clear	++	Transparent	23.1±0.6	49.8±0.5
F6	7.43±0.13	Clear	+++	Transparent	29.8±0.3	56.3±0.8

Mean ±SD (Average of 3 readings), +: gels after few min and dissolves within 45 min. ++: gelation immediate, remains for few hrs. +++: gelation immediate, remains for extends period.

In vitro drug release study

In vitro release data revealed that an increase in the polymer content was associated with a slow and extended release of drugs. Drug release is also depending on the type of polymer used. Here carbopol 934 p was used as gelling agent and HPMC as viscosity enhancer. This study showed that high concentration of polymers controls the delivery of drugs. The hydrogel provides sustained release of drug up to 75 – 80% at the end of 9 h. The *in vitro* studies revealed that *in situ* hydrogel was found to better sustain the release of the drugs in the following order F2>F3>F1>F4>F5>F6. Formulation F6 showed more sustained release compared to the other formulation. This could be the reason of higher concentration carbopol 934 p and HPMC among the developed formulation and there was slow and prolonged release. *In vitro* drug release for all the formulations were shown in Figure 2- A**:** % cumulative drug release of OFL, B: % cumulative drug release of DX by plotting cumulative % drug release v_s time. Song *et al.,* 2013 reported that *in vitro* drug release is mainly dependent on two processes i.e., water migration into the *in situ* gelling system and drug diffusion. The prolonged release may be probably due to the formation of hydrogen bonds between drug and polymers, which have helped in sustained release of drug. The release of drug from all the formulations followed zero order kinetics.

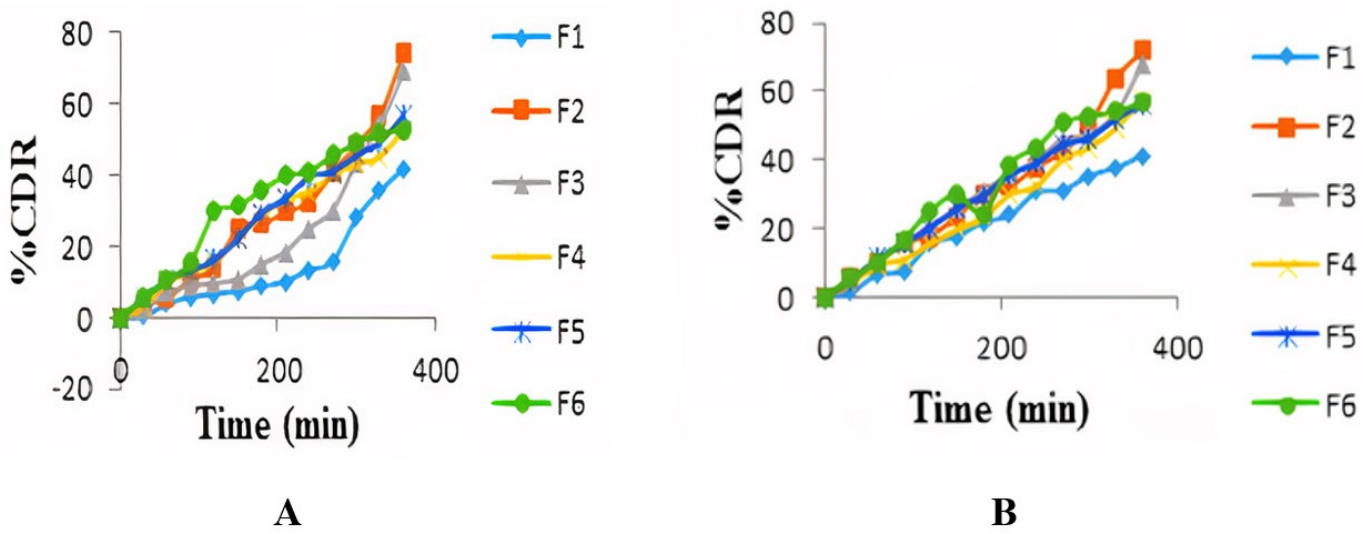

FIGURE 2 - (A) - % cumulative drug release (CDR) of OFL. **(B) -** % cumulative drug release (CDR) of DX.

Kinetic analysis of *in vitro* release data

Release mechanism mainly provides the best description to the pattern of drug release, the *in vitro* release data were fitted to zero order, first order, and higuchi model and also data were also kinetically analyzed using the Korsmeyer –Peppas model. By using MS-EXCEL statistical function data were analyzed. In the present study, the release mechanism followed the combination of diffusion and erosion as the 'n' values ranged from 0.519 to 0.824 for OFL and for DX values ranged from 0.514 to 0.812 for as per Korsmeyer and Peppa's model. Kinetic analysis of *in vitro* results showed that, all the formulations fitted in to zero order kinetic model and r^2 values in the range between 0.9521 to 0.9841 for OFL and for DX 0.9687 to 0.9878 (Table III).

TABLE III - Kinetic analysis of *in vitro* drug release data of OFL and DX

Formulation Code	Zero order (R^2)		First order (R^2)		Higuchi model (R^2)		Korsmeyer Peppas Model (R^2)		Best fitting model
	OFL	DX	OFL	DX	OFL	DX	OFL	DX	
F1	0.9521	0.9823	0.9312	0.9813	0.9256	0.9126	0.554	0.812	Zero
F2	0.9841	0.9878	0.9346	0.9586	0.9360	0.9236	0.721	0.756	Zero
F3	0.9756	0.9758	0.9681	0.9666	0.9638	0.9425	0.524	0.683	Zero
F4	0.9491	0.9687	0.9529	0.9526	0.9553	0.9563	0.824	0.514	Zero
F5	0.9630	0.9864	0.9784	0.9645	0.9756	0.9125	0.519	0.624	Zero
F6	0.9568	0.9782	0.9654	0.9771	0.9364	0.9356	0.501	0.763	Zero

Test for sterility

The test was performed as per the literature. Both positive and negative controls were prepared. The results of the sterility when compared with positive and negative control showed that the medium used was sterile and provided necessary nutrients for the microorganism. Further, it could also be interpreted that the presence of drugs did not show any antimicrobial or antifungal activity since the growth of organism is found to be equal in growth promotion test (positive control) and test for bacteriostasis/fungistasis test. The results for the test for sterility are given in Table IV. After examination, there was no macroscopic evidence of microbial growth. Hence, it passes the test for sterility.

TABLE IV - Test for sterility in fluid thioglycolate medium (F) and soyabean casein digest medium (S)

Days	1		2		3		4		5		6		7	
	F	S	F	S	F	S	F	S	F	S	F	S	F	S
Formulations	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Positive control	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Negative control	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+): Growth of microorganism; (-): No growth

Anti-microbial activity

The antimicrobial activity was determined by an antibiotic zone reader. The zones of inhibition were measured at 24h. The zone of inhibition was found to be in between 26-28 mm. Antimicrobial efficacy study was performed for all formulation using *Staphylococcus aureus*. The zone of inhibition for F2 formulation was 28 mm and for standard solution was 28.7 mm. The results of antimicrobial activity are as shown in the (Table V) and (Figure 3 A, B & C). The study indicated that prepared formulation retained its antimicrobial activity when formulated as *in situ* hydrogel against *Staphylococcus aureus*.

TABLE V - Antimicrobial activity test

Microorganism	Conc µg/mL	Zone of inhibition(mm)					
		F1	F2	F3	F4	F5	F6
Staphylococcus aureus	2	27±0.3	28±0.2	27.5±0.2	27.5±0.5	27±0.6	26.5±0.4

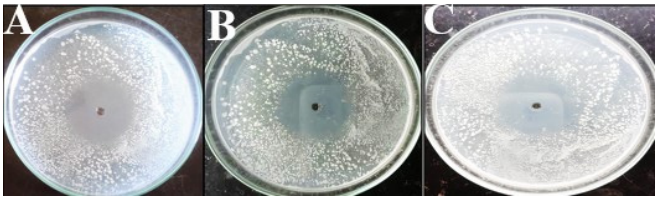

FIGURE 3 - (A)- Zone of inhibition of F2 formulation. **(B) -** Zone of inhibition of F4 formulation. **(C) -** Zone of inhibition of F6 formulation.

Ocular irritation studies

The formulations F2 and F6 were selected for the ocular irritation study. No ocular damage, irritation to the cornea, iris or conjunctiva was observed. Selected formulations were nonirritant to the eye and excellent ocular tolerance was noted. These results indicated that the prepared formulation safe to use for further *in vivo* studies to evaluate the effect of OFL and DX *in situ* hydrogel activity for the treatment of conjunctivitis.

In vivo studies

In the present study, 150 mg of OFL and 50 mg of DX was used for the preparation of *in situ* hydrogel. For *in vivo* study, the drug concentration of drug was selected in such a way that the drug concentrations when administered into the eye, -is comparable with the eye drops (Oflox-D, each mL contains OFL- 0.3 % w/v & DX- 0.1 %w/v) i.e., each drop of marketed formulation contains approximately 15 mg of OFL and 5 mg of DX, hence in the present study the formulation was prepared such that it contains approximately 15 mg of OFL and 5 mg of DX.

Criteria of conjunctivitis response to drug therapy

Decrease in redness, mucoid discharge, lachrymal secretion, response to ocular stimulus were taken as positive response to therapy (Figure 4 A, B & C). So, *in situ* hydrogel formulation was effective in relieving symptoms of conjunctivitis with the advantage of lesser frequency of administration compared to eye drops which are usually instilled into the eye at a frequency rate of 2-3 times a day.

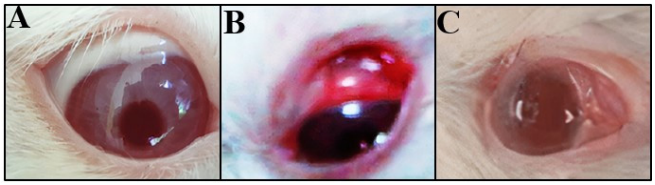

FIGURE 4 - (A) - Rabbit eye without infection (Normal eye). **(B) -** Day 2 recovery after administration marketed formulation. **(C) -** Day 2 recovery after administration of *in situ* hydrogel.

Stability studies

Stability studies of optimized formulations were carried out for 60 days. The *in situ* hydrogel was observed for physical change, percentage drug release. *In situ* hydrogel containing a combination of OFL and DX was found to be physically and chemically stable and showed no significant change in terms of physical characteristics and percentage drug release. All the formulations showed good stability in all the condition. The results of stability studies for optimized formulation were shown in Table VI.

TABLE VI - Stability test

Test Parameters	Results
Clarity	Clear
Gelling capacity	+++
pH	7.39±0.13
Viscosity	35.9±0.2
In vitro drug release	77.6 %

CONCLUSION

In the present study *in situ* hydrogel of ofloxacin and dexamethasone for the treatment of conjunctivitis was successfully formulated and evaluated. The developed Carbopol 934p and HPMC based *in situ* hydrogel formulation prolongs residence time of the drug at the site of application, and in turn has better therapeutic effect. A considerably low drainage of ofloxacin and dexamethasone into circulation as compared to eye drops is an added advantage in the treatment of conjunctivitis. Thus, based upon obtained results it can be concluded that the F2 formulation has shown more than 85 % of drug released in 13 h. Moreover, this novel formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer pre-corneal residence time and ability to sustain drug release. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance. In order to maximize the potential

of this system for ocular drug delivery, further studies are needed to examine its feasibility for the prolonged delivery of other ocular drugs.

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