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FORMULATION AND EVALUATION OF IN SITU GEL OF LEVOFLOXACIN AND DEXAMETHASONE

Prashant Pachori*, Aditya Tiwari and Nitendra Sahu

Millinnium College of Pharmacy, Bhopal.

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***Corresponding Author**
Prashant Pachori
Millinnium College of Pharmacy, Bhopal.

ABSTRACT

In-situ gel of Levofloxacin and dexamethasone was formulated and evaluated. During the preformulation, the solubility, Melting point, Wavelength of maximum absorption (λmax) and Calibration curves were obtained of Levofloxacin and Dexamethasone. Gellan based *in situ* gel systems were prepared by dissolving gellan alone and its combination with sodium alginate and/or HPMC in hot phosphate buffer mannitol (5% m/V) were added as isotonicity agent. Different evaluations were carried out as visual appearance, transparency, clarity, pH, drug content, viscosity, in-vitro gelation studies, gel strengths, bioadhesion, and sterility, all evaluated parameter are under limit as required for *in-situ* gel formation. Among the all prepared L5 has drug contain above 99% of both drugs gelation immediate and remains for an extended period, Gel Strength (peak Load) of 72g and best bio-adhesion of 35g. %Cumulative drugs release from formulation L5 in STF solutions was studied where 69.72% Levofloxacin and 72.34% Dexamethasone was released. The ophthalmic formulations must be isotonic to avoid damage to delicate tissues of eye. It is concluded that the optimized in-situ gel formulation is a better in results and evaluation parameters.

KEYWORDS: *In-situ* gel, Levofloxacin, Dexamethasone, Isotonicity, Bioadhesion.

INTRODUCTION

In situ gelling systems are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physicochemical parameter (Ionic strength, Temperature, pH).[1]

They are highly advantageous over preformed gels as they can easily be applied in liquid

form but are capable of prolonging residence time of the formulation on the surface due to gelling.[2] The principal advantage of *in situ* gelling systems is easy, accurate, and reproducible administration of a dose compared to application of preformed gels.[3] Ophthalmic *In-situ* gels are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physicochemical parameter like ionic strength, pH or temperature.[4] It provide targeting within the ocular globe so as to prevent the loss to other ocular tissues.[5] In situ gelling approach amalgamates the advantages of both solutions and gels, such as accuracy and facility of administration of the former and prolonged residence time of the latter.[6] In situ forming gels are liquid upon instillation and undergo phase transition in the ocular cul-de- sac to form viscoelastic gel and this provides a response to environmental changes.[7] Levofloxacin is used to treat infections of the sinuses, skin, lungs, ears, airways, bones, and joints caused by susceptible bacteria.[8] Levofloxacin is frequently used to treat urinary infections, including those resistant to other antibiotics, as well as prostatitis (infection of the prostate).[9] Dexamethasone is used to treat conditions such as arthritis, blood/hormone/immune system disorders, allergic reactions, certain skin and eye conditions, breathing problems, certain bowel disorders, and certain cancer.[10]

Formulations of Levofloxacin and Dexamethasone are available in the form of eye drops and eye ointments in the market.[11] When administered as eye drops, many reports were found regarding poor bioavailability because of solution drainage, rapid precorneal elimination and tear turnover.[12] When ointment is applied topically to the cornea, blurred vision is the major problem, which may result in reduced patient compliance.[13] This leads to frequent instillation of concentrated medication to achieve the desired therapeutic effect. Therefore it is necessary for research scientists to develop an ocular drug delivery system which will overcome and reduce side-effects associated with conventional ocular preparations with better bioavailability.

The aim of this research was to develop various novel ophthalmic controlled drug delivery systems like ion activated *in situ* gel, for some antibacterial fluoroquinolones like Levofloxacin and Dexamethasone for the effective treatment of various ocular disorders like Glaucoma, conjunctivitis, corneal ulcer, trauma.

MATERIAL AND METHOD

Preformulation studies

Solubility studies: A fixed amount of drug was taken then solvent e.g. water, methanol, 0.1N NaOH, 0.1N HCl, Ethyl acetate was added and observes the solubility visually. The solubility of the drug was obtained by dissolving amount of drug in individual solvent to the saturation and then concentration of prepared solution was measured using UV spectrophotometer.

Melting point: The Melting point was determined by the capillary method using Digital Melting point apparatus. The capillary tube was fused and filled by pressing the open end gently into pure drug sample and packed by tapping the bottom of the capillary on a hard surface so that the drug packed down into the bottom of the tube. When the drug was packed into the bottom of the tube, the tube was placed into the slot of the apparatus, the apparatus was started and the temperature was noted at which the drug melt.

Preparation of tear fluid: Simulated tear fluid (STF) was prepared by dissolving sodium chloride (0.67g), sodium bicarbonate (0.20g), calcium chloride dihydrate (0.008g) in distilled water q. s. 100 ml. prepared solution was stored in volumetric flask.

Determination of wavelength of maximum absorbance (λ_{max})

10 mg of drug was weighed accurately and transferred to 10 ml of volumetric flask then STF, was added to dissolve the drug completely. The volume was made up to 10 ml with STF, The prepared sample was 1000 µg/ml. 1ml of above solution was then transferred to another 10 ml volumetric flask and diluted it upto the mark with STF results 100 µg/ ml. 1ml of above solution was then transferred to another 10 ml volumetric flask and diluted it upto the mark with STF results 10µg/ ml. This concentration was scanned from 200-400nm by UV-spectrophotometer.

Preparation of calibration curve

(a) Stock solution: Pure drug **(**Levofloxacin or Dexamethasone) (10 mg) was dissolved in 10ml of STF in 10 ml volumetric resulted solution was 1000µg/m. this prepared solution was stock solution, it is used for preparation of various dilutions.

(b) Preparation of dilutions: From this stock solution, aliquots of 2, 4, 6, 8 and 10 µg/ml concentration was prepared form stock solution using serial dilution method. The absorbance of these solutions was taken by double beam U.V. spectrophotometer using particular λ_{max} of drug.

(c) Preparation of calibration curve: The absorbance values of different dilutions were plotted against respective concentration (µg/ml) and obtained the standard calibration curve. MS- excel tool was used to prepare calibration curve, linearity equation and regression (R^2) value.

Drug-Excipients Compatability Study FT-IR: FT-IR Spectroscopy can be used to investigate and predict any physicochemical interactions between different components, in a formulation and therefore it can be applied to selection of suitable chemically compatible excipients. While selecting the ingredients, we would choose those which are stable, compatible and therapeutically acceptable. The aim of compatibility study was to test, whether there is any interaction between the excipients and the drug and compatibility between the drug and excipients.

Method of formulation of *in-situ* gelling systems for ophthalmic drug delivery

Selection of Method and Polymers

Preliminary studies were carried out to select a suitable polymer system, which is capable of producing *in situ* ophthalmic gels of desirable physical property. Different formulations were prepared with the use of polymers like Gelrite® gellan gum - Kelcogel F, Sodium alginate, poloxamer 188, poloxamer 407 and Carbopol 940P. Gellan gum and Sodium alginate were meant for electrolyte triggered *in situ* gelling system, and Carbopol 940p was meant for pH sensitive *in situ* gelling system. These preliminary studies were carried out to find out the suitable proportion of polymer blends to be used for the manufacturing of *in situ* ophthalmic gel and to derive the range of polymer blends for desired property.

Method of formulation of *in-situ* gel

Gellan based *in situ* gel systems were prepared by dissolving gellan alone and its combination with sodium alginate and/or HPMC F4M in hot phosphate buffer (70˚C, prepared in fresh water for injection under laminar flow) pH 7.4, with or without different proportions of sodium citrate, by continuous stirring at 40-45˚C for 24 hr. Then the weighed quantities of Levofloxacin required to give final drug concentration of 0.5% w/v, and dexamethasone in 0.1%, benzalkonium chloride (0.5% m/v) were added to the polymeric solution and stirred until dissolved, mannitol (5% m/V) were added to the polymeric solution and stirred until dissolved. The solutions were transferred into previously sterilized amber colored glass vials. The formulations were sterilized by terminal autoclaving at 121˚C and 15p.s.i. for 20 minutes. The sterilized formulations were stored in a refrigerator

(4-8˚C) until use.

Evaluation and Characterization of prepared batches

Visual Appearance: Prepared formulations were subjected for visual inspection like color consistency transperancyetc.

Clarity test: Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background. pH is also one of the most important parameters involved in the ophthalmic

Determination of pH: The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4. The pH was measured for each formulation using a pH meter, which was calibrated before use with buffered solutions of pH 4 and 7.

Drug content: Uniform distribution of active ingredient is important to achieve dose uniformity. The vials (n=3) containing the preparation were shaken for 2-3 minutes and 100 µl of the preparation was transferred aseptically to sterile 25-ml volumetric flasks with a micropipette and the final volume was made up with phosphate buffer pH 7.4. The concentration of Levofloxacin and Dexamethasone was determined at 286 nm and 244 nm respectively.

Tonicity test: Tonicity of the formulation was determined using Rat blood. The principle that blood cells shrinks in hypertonic solution, they raptures in hypotonic solution and retain its shape in isotonic solution (normal saline) was utilize. Effect of formulation with isotonic agent (5% mannitol), and formulation without isotonic agent on blood cells was measured and comparedwith blood cells in normal saline.

Sterility test: Direct inoculation method was used to perform the sterility of the prepared formulations. From the test solution (2 ml) was withdrawn using a sterile pipette and aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean - casein digest medium (20 ml) separately. After inoculation the media was incubated for not less than 14 days at 30-35 °C in the case of fluid thioglycolate medium and 20-25 °C in the case of soyabean-casein digest medium.

***In-vitro* gelation studies:** The gelation studies were carried out in a vial containing simulated tear fluid (STF solution) as gelation solution. The composition of STF was sodium chloride

0.670 g, sodium bicarbonate 0.200g, calcium chloride dihydrate 0.008g and purified water q.s. to 100 g, which stimulate either the divalent cation content or both the divalent cation content and protein of the tear fluid. The preparations (100 µl) were carefully placed into the vial using a micropipette, and 2 ml of gelation solution (STF) was added slowly. Gelling capacity was examined by visually assessing the gel formation and noting the time for gelation and the time taken for the gel formedto dissolve.

Rheological studies: The viscosity of the prepared formulations was determined at different angular velocities at 34±1˚C using small volume adaptor of the Brookfield Viscometer (LV model). A typical run involved changing the angular velocity from 0.5 to 100 rpm at a controlled ramp speed. After 6sec at 0.5 rpm, the velocity was successively increased to 100 rpm, with a similar period at each speed. The angular velocity was then reversed (100-0.5 rpm) for a similar period of 6 sec.[18] The average of two readings was used to calculate the viscosity. To evaluate the viscosity change after administration, rheological measurements were taken after diluting the formulations with STF[16] in 25:7 ratio (application volume, 25 µl; normal volume of tear fluid in the eye, 7 µl).

Gel strength determination: Gel strength was determined using a Brookfield Texture Analyzer (USA) in compression mode. Formulations with STF (50+14 ml) were transferred into cylindrical holder shown in Figure 4.7 (a) & (b) (Refer Chapter 4, Section 4.4.2), gel strength and bioadhesion measurement respectively, taking care to avoid the introduction of air into the samples. A cylindrical analytical probe (38 mm diameter) was forced down into each sample at a defined rate (30 mm/min) and toa defined depth (10 mm). At least three replicate analyses of each sample were performed with the simulated tear fluid. From the resulting load–time plots, the gel strength (the maximum force required to attain a given deformation i.e. peak load) and adhesive force (the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe) were derived.

***In-vitro* drug release studies:** The studies were carried out using Franz diffusion cell with STF (pH 7.4) as dissolution medium. The cell consisted of glass donor and receptor compartment. The prepared formulations were placed in donor compartment and freshly prepared STF in receptor compartment. Between donor and receptor compartment diffusion membrane was placed. The whole assembly was placed in the thermostatically controlled shaker water bath. The temperature of the medium was maintained at 37˚C± 0.1˚C. 1ml of sample was withdrawn at predetermined time interval and same volume of fresh medium was replaced. The withdrawn samples were diluted and analyzed by UV spectrophotometer.

Stability studies

Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. Stability studies were carried out on optimized formulation according to ICH guidelines. A sufficient quantity of formulation in amber-colored vials was stored in stability chamber at ambient humidity conditions at 2˚C to 8˚C, ambient temperature at 40±0.5˚C for period of 6 months. The samples were withdrawn at 0, 30, 60, 90, and 180 days and analysed for key parameters like gelling capacity, pH, viscosity, *in vitro* drug release etc. The logarithms of percent drug remaining were calculated and plotted against time in days. The degradation rate constant was calculated with equation slope = k/2.303, where k is a degradation rate constant. The shelf life of the developedformulation was calculated using the Arrhenius plot.

RESULTS

During the preformulation it was observed that the Levofloxacin is yellowish colored, bitter, odorless crystalline powder and Dexamehasone was white, Slightly bitter, odorless, Crystalline powder. A fixed amount of drug was taken to determine solubility in water, methanol, 0.1N NaOH, 0.1N HCl, Ethyl acetate and found that Levofloxacin soluble in 0.1N NaOH, while Dexamethasone soluble in all solvents. The Melting point was found as 220-2220C and 261-262 0C for levofloxacin and dexamethasone respectively. Wavelength of maximum absorption (λmax) of levofloxacin and Dexamethasone was determined at 286 nm and 244nm respectively. Calibration curves of Levofloxacine and Dexamethasone were prepared drugs in Simulated Tear Fluid with r^2 value 0.999 for both drug. FTIR spectra of both drug shows no chemical interactions.

Table 1: Calibration curve of pure drugs in simulated tear fluid.

Conc. (µg/ml)	0	2	4	6	8	10
Levofloxacine (286nm)	0	0.202	0.378	0.552	0.723	0.916
Dexamethasone(244nm)	0	0.198	0.354	0.534	0.700	0.886

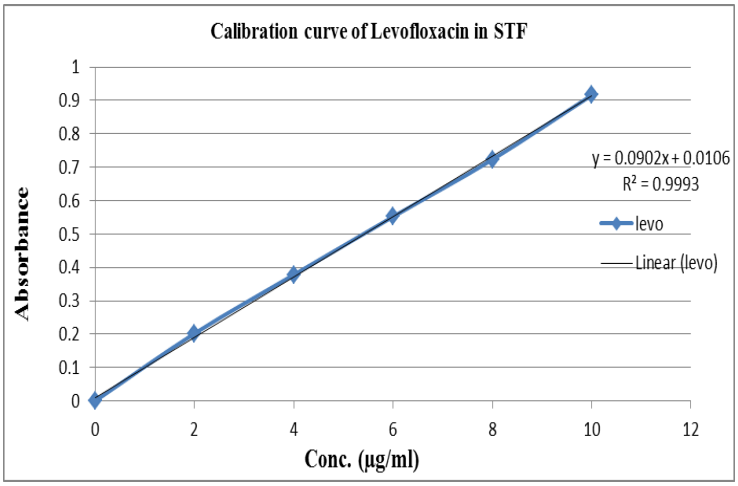

Figure 1: Calibration curve of levofloxacin in STF.

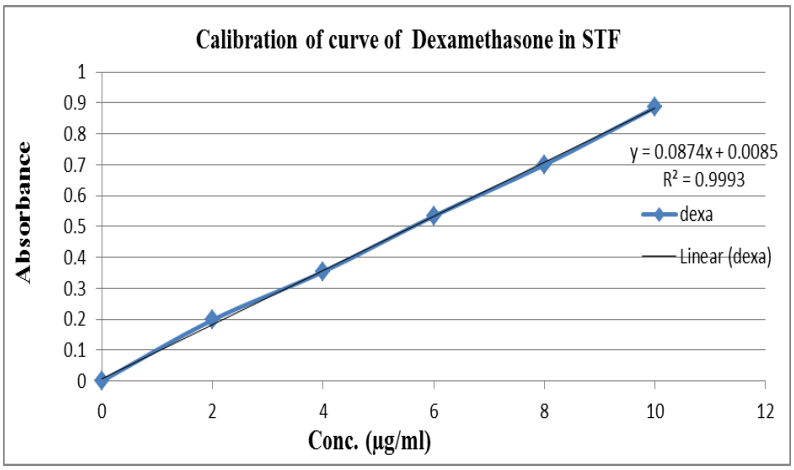

Figure 2: Calibration curve of Dexamethasone in STF.

Formulation of *in-situ* gelling systems for ophthalmic drug delivery

Gellan based *in situ* gel systems were prepared by dissolving gellan alone and its combination with sodium alginate and/or HPMC in hot phosphate buffer (70˚C, prepared in fresh water for injection under laminar flow) pH 7.4, with or without *different* proportions of sodium citrate, by continuous stirring at 40-45˚C for 24 hr. Then the weighed quantities of Levofloxacin required to give final drug concentration of 0.5% w/v, and dexamethasone in 0.1%, benzalkonium chloride (0.5% m/v) were added to the polymeric solution and stirred until dissolved. The solutions were transferred into previously sterilized amber- colored glass vials. The formulations were sterilized by terminal autoclaving at 121˚C and 15p.s.i. for 20 minutes. The sterilized formulations were stored in a refrigerator (4-8˚C) until use.

Table 2: Composition of in-situ ophthalmic gel preparations.

Batches	L1	L2	L3	L4	L5
Levofloxacin	0.5%	0.5%	0.5%	0.5%	0.5%
Dexamethasone	0.1%	0.1%	0.1%	0.1%	0.1%
Gellan (%w/v)	0.3	0.3	0.3	0.3	0.3
Na Alginate (%w/v)	---	0.3	0.4	-	---
HPMC F4M (%w/v)	---	---		0.3	0.5
Phosphate buffer pH 7.4 (ml)	100	100	100	100	100

Evaluation and Characterization of prepared batches

Table 3: Results of evaluation of prepared batches.

Evaluation parameter	L1	L2	L3	L4	L5
Visual appearance	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Light Yellow
Transparency	**T**	**T**	**T**	**T**	**T**
Clarity	**Clear**	**Clear**	**Clear**	**Clear**	**Clear**
pH	6.2	6.1	6.1	6.0	6.0
Drug content (Levofloxacin)	98.0 ± 0.4	98.1 ± 0.5	99.1 ± 0.2	98.7 ± 0.1	99.2 ± 0.3
Drug content (Dexamethasone)	98.4 ± 0.4	98.3 ± 0.5	99.0 ± 0.2	98.8 ± 0.1	99.5 ± 0.3
Viscosity (cps)	300	350	478	452	398
In-vitro gelation studies	++	+	++	++	+++
Gel Strength (peak Load)	40g	49g	54g	60g	72g
Bio adhesive	15 g	29 g	19 g	22 g	35 g

Note: + gels slowly and dissolves; ++ gelation immediate and remains for a few hours; +++ gelation immediate and remains for an extended period. T= Transparent

***In-vitro* drug release studies**

Table 4: %Cumulative drugs release from formulation L5 in STF solution.

Time	Levofloxacin	Dexamethasone
0	0.00	0.00
1	7.55	8.01
2	15.19	17.34
3	24.58	27.42
4	33.68	36.08
5	44.34	48.02
6	52.80	55.02
7	62.52	64.61
8	69.72	72.34

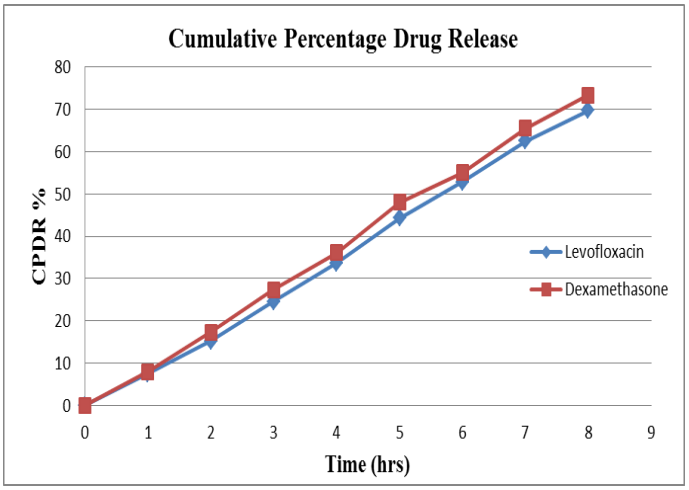

Figure 3: *In vitro* release profiles of batches L 5.

Tonicity determination

The ophthalmic formulations must be isotonic to avoid damage to delicate tissues of eye. Effect of formulation with isotonic agent (5% mannitol), and formulation without isotonic agent on blood cells was measured and compared with blood cells in normal saline using microscope, it is proved that the solution is isotonic with the body fluid.

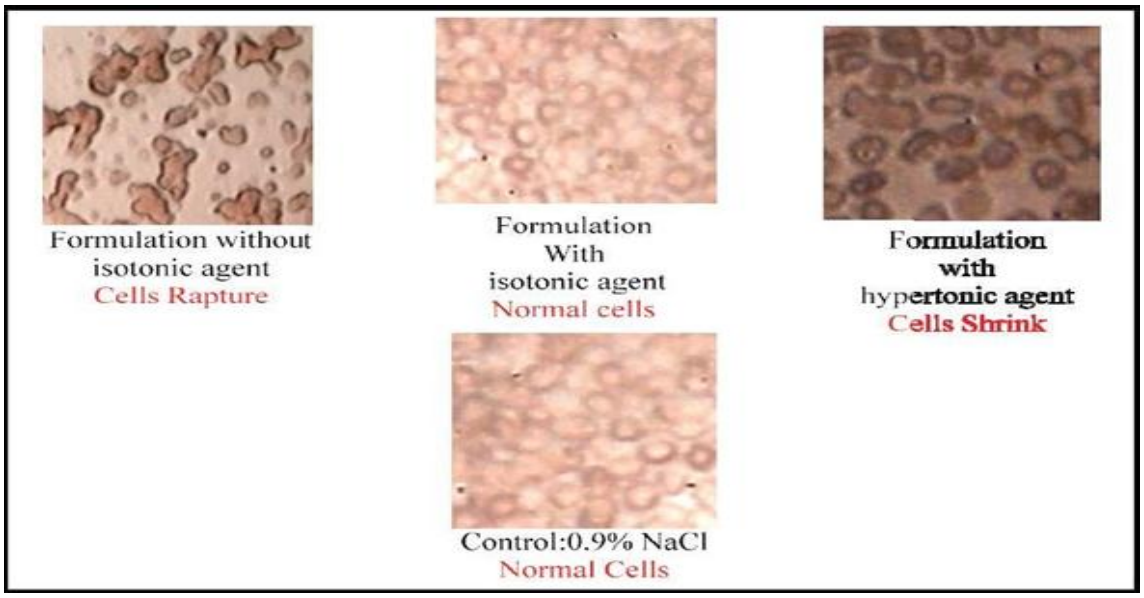

Figure 4: Shape of blood cells after addition of formulation.

Stability studies

The formulation was found to be sterile at the end of 01 months. Drug degraded to a negligible extent and the degradation rate constant for optimized formulation was very low (1.12×10^{-4}). Because the overall degradation is <5%, a tentative shelf life of 2 years may be assigned to the formulation. The similarity factor F_2 was calculated for checking similarity

between optimized batch L5 and stress batch L'5 (after 01 months stability period). The F_2value was 83.57, indicating that the both batches are similar. (F_2 value more than 50, indicated batches are similar).

Table 5: Stability study of optimized batch L5 and LF5' (after 01 months).

Time (Hrs.)	L5 (before)	L'5'(After 01 months)
0	0	0
1	7.61±0.2	6.51±0.2
2	15.30±0.5	14.2±0.1
3	24.63±0.6	23.22±0.3
4	35.45±0.3	33.78±0.5
5	46.96±0.4	44.54±0.1
6	57.96±0.5	55.99±0.2
7	65.43±0-.6	63.82±0.5
8	74.86±0.7	71.98±0.1

DISCUSSION

In-situ gel of Levofloxacin and dexamethasone was formulated and evaluated. During the preformulation it was observed that the Levofloxacin is yellowish colored, bitter, odorless crystalline powder and Dexamehasone was white, Slightly bitter, odorless, Crystalline powder. A fixed amount of drug was taken to determine solubility in water, methanol, 0.1N NaOH, 0.1N HCl, Ethyl acetate and found that Levofloxacin soluble in 0.1N NaOH, while Dexamethasone soluble in all solvents. The Melting point was found as 220-2220C and 261-262 0C for levofloxacin and dexamethasone respectively. Wavelength of maximum absorption (λmax) of levofloxacin and Dexamethasone was determined at 286 nm and 244nm respectively. Calibration curves of Levofloxacine and Dexamethasone were prepared drugs in Simulated Tear Fluid with r^2 value 0.999 for both drug. FTIR spectra of both drug shows no chemical interactions.

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ophthalmic formulations must be isotonic to avoid damage to delicate tissues of eye. It is concluded that the optimized in-situ gel formulation is a better in results and evaluation parameters.

CONCLUSION

Levofloxacin, a broad-spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as ion-activated *in situ* gel-forming ophthalmic solutions with dexamithasone drug (0.5% (w/v)) using Gelrite® gellan gum as a gelling agent in combination with HPMC as a viscosity-enhancing agent. Combining gellan with sodium alginate did not offer any advantage(as in therapeutic efficacy) over the formulations based on gellan alone. The formulation underwent gelation in the cul-de-sac upon instillation as drops into the eye. The gel formed *in vitro* produced sustained drug release over 8-h period and the developed formulations were devoid of any deleterious effect on the ocular tissues. The formulations demonstrated better therapeutic efficacy as they were successful in inhibiting the growth of the microorganisms for the entire duration of the study when compared with the marketed eye drop. Stability data recorded over a 6-month period under accelerated temperature conditions indicated that the formulation is stable. This new formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its sustained drug release, higher viscosity, longer pre-corneal residence time, and better miscibility with the lachrymal fluid. Also important is its ease of administration and reduced frequency of administration resulting in better patient acceptance and compliance.

Conflicts of interest

There are no conflicts of interest.

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