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Original Article

OPTIMIZATION AND CHARACTERIZATION OF ION ACTIVATED OCULAR *IN-SITU* GEL FORMULATION FOR BACTERIAL CONJUNCTIVITIS

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ABSTRACT

Objective: The present research work aims at describing the formulation, optimization and evaluation of ion activated ocular *in-situ* gel of gatifloxacin for treatment of bacterial conjunctivitis so as to overcome patient inconvenience, precorneal drug elimination, variation in efficacy, vision blurring and frequent instillation associated with conventional eye drops and ointments.

Methods: *In-situ* gel was prepared using *gellan gum* as an ion activated phase transition polymer and HPMC K100M as release retardant. Gatifloxacin was characterized by spectrophotometry. Crystalline state of the drug was determined using X Ray Diffraction study. The developed formulation exhibited instantaneous gel formation in simulated lacrimal fluid (pH 7.4), which was further evaluated for its rheology, irritancy parameters, *in vitro* release, trans-corneal permeation and antimicrobial activity.

Results: Gatifloxacin exhibited λ_{max} 286 nm obeying Beer Lambert's law and pH-dependent solubility at a pH range of 2 to 4. 0.6% *gellan gum* and 0.4% HPMC K100M were optimized in the formulation which exhibited a viscosity of 55 cps in sol form and 325 cps in gel form with pseudoplastic behavior and prolonged *in vitro* release. Permeation of formulation was 75.8% in 7 h with log P of drug 0.59. Developed isotonic and non-irritant formulation had a lower apparent permeability coefficient of 8.15×10^{-5} cm/sec as compared to marketed formulation.

Conclusion: A Formulation can be viewed as an efficacious medicine by virtue of its higher zone of inhibition, ability to enhance precorneal residence time and consequently ocular bioavailability with lesser frequency of administration attributed to slow and prolonged diffusion of the drug from the polymeric solutions.

Keywords: Gatifloxacin, *In-situ*, Pseudo plastic, Pre-corneal Residence, Isotonic, Rheology, On activated

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INTRODUCTION

The term "conjunctivitis" encompasses a broad group of conditions referred as inflammation of the conjunctiva. The inflammation can be hyperacute, acute or chronic and infectious or noninfectious in origin. Conjunctivitis is the most common cause of red-eye. Most frequently, conjunctivitis (and thus red eye) is caused by bacterial or viral infection [1]. Bacterial conjunctivitis is usually caused by *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus* species, *Pseudomonas aeruginosa*, and *Chlamydia trachomatis* [2-4]. Topical administration of eye drops in the lower cul-de-sac is the most common method of drug delivery for the treatment of ocular disease [5]. However, one of the major problems encountered with the eye drops is the rapid and extensive elimination induced by tear turnover, blinking, and drainage of formulation, which leads to short precorneal residence time and poor ocular bioavailability. As a result, frequent instillation of eye drops is needed in order to achieve the desired drug concentration and therapeutic effect [6]. An increase in dosing frequency or use of the highly concentrated solution to compensate for short ocular residence time is undesirable because of poor patient compliance and risk of toxicity due to ophthalmic absorption via the nasolacrimal duct [7]. To increase ocular bioavailability and duration of drug action, various ophthalmic vehicles, i.e., viscous solutions [8], ointments/gels [9], and polymer inserts [10] have been used. These ocular drug delivery systems, however, have not been used extensively owing to some drawbacks, such as blurred vision from ointments, lack of patient compliance from inserts and sticking of eyelids from the gel.

An ideal ophthalmic dosage form is one that can sustain the drug release and remain in pre-corneal contact for an extended period of time. A significant increase in the residence time of the formulation and consequently, drug bioavailability can be achieved by delivery systems based on the concept of *in-situ* gelation [11]. These delivery systems consist of polymers that exhibit sol to gel phase transition due to changes in specific physiological conditions (pH, temperature and ionic strength) in the eye [12]. Depending upon the method employed to cause a phase transition on ocular surface, three types of systems are recognized, i.e., pH triggered systems-cellulose acetate hydrogen phthalate latex [13] and Carbopol [14-16], temperature-dependent systems-pluronic [17-19] and tetronics [20] and ion activated systems-*gellan gum* [21, 22] and *sodium alginate* [23]. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition, once administered.

Gatifloxacin is a fourth-generation flouroquinolone antibiotic used for the treatment of bacterial conjunctivitis. It is commercially available in the form of an eye drops and ointment. The topical ophthalmic administration of 0.3% gatifloxacin solution is indicated in case of severe infection.

The objective of the present work involved the development of an *in-situ* gel formulation using an ion-activated phase transition polymer to effectively deliver the drug into the eye for sustained drug release and enhanced ocular drug bioavailability.

MATERIALS AND METHODS

Materials

Gatifloxacin was used as an active pharmaceutical ingredient and was procured as a gift sample from M/s. Alkem Laboratories Ltd (Mumbai, India). *Gellan gum* and HPMC were procured from Chempure (Mumbai, India) and Colorcon Asia Pvt. Ltd. (Mumbai, India), respectively. The nutrient agar medium was purchased from Hi-Media (India). Microbial strains were available at ISF Moga to perform the study, which was procured from MTCC Chandigarh. Whole eyeballs of goat were procured from the slaughter house. All the other reagents were used in the present study were of analytical grade.

Preformulation studies

Determination of λ_{max} by UV spectrophotometer

One hundred mg of gatifloxacin drug powder was accurately weighed and transferred to a 100 ml volumetric flask. It was

dissolved in an adequate amount of distilled water and the volume was made up to 100 ml with distilled water so as to obtain a stock solution of 1000 µg/ml [24]. A dilution of 20 µg/ml concentration was made from the above stock solution with the distilled water and the resulting solution was scanned on a double-beam UV-visible spectrophotometer (Shimadzu 1700) between wavelength ranges of 200 nm to 400 nm [25].

Infrared spectral analysis of gatifloxacin drug sample

Gatifloxacin powder sample was compressed into a pell*et al.* ong with KBr (KBr pellet technique) using a shimadzu hydraulic press [26]. The FTIR spectrum of the drug was recorded between the wavenumber regions of 500-4000 cm^{-1} on an FTIR spectro-photometer (Shimadzu) [27].

Melting point determination

The melting point of gatifloxacin was determined using the open capillary method. The drug sample was filled into a capillary and placed in a Thiel's tube filled with liquid glycerol. The tube was heated and the temperature at which the drug melted was noted.

X-ray diffraction study of gatifloxacin drug sample

X-ray diffraction analysis was performed using Rigaku diffractometer (high beam monochromatic) using Cu Kα radiation, which was generated at 40 Kv and 100 mA at λ 1.5418Å. The data were collected over an angular range from 5-30º in a continuous mode.

Analytical method development

Preparation of calibration curve of gatifloxacin in distilled water

Ten mg of gatifloxacin drug sample was accurately weighed and transferred into a 100 ml volumetric flask. It was dissolved in an adequate amount of distilled water and the volume was made up to 100 ml with distilled water to obtain a stock solution of 100 µg/ml. Appropriate dilutions were made in distilled water from the above stock solution in the concentration range of 2-14 µg/ml and were analyzed spectrophotometrically at 286 nm against a blank prepared in the same manner. The absorbance data for different concentrations were subjected to regression analysis.

Preparation of calibration curve of gatifloxacin in simulated lacrimal fluid

Ten mg of gatifloxacin drug sample was accurately weighed and transferred into a 100 ml volumetric flask. It was dissolved in an adequate amount of distilled water and the volume was made up to 100 ml to obtain a stock solution of 100µg/ml. Appropriate dilutions were made in simulated lacrimal fluid from the above stock solution the concentration range of 2-14 µg/ml and were analyzed spectrophotometrically at 286 nm against a blank prepared in the same manner. The absorbance data for different concentrations were subjected to regression analysis.

Partition coefficient (Log P) determination

The partition coefficient (P) is the quotient of two concentrations and is usually given in the form of its logarithm to base 10 (log P). Partition coefficient of the gatifloxacin drug sample was determined by saturating 10 ml of octanol with 10 ml of distilled water for 30 min. Twenty mg of the drug was added to this blend and was moderately shaken for 1 hour with 5 min interval. The two solvent layers were separated through separating funnel and separately filtered through a Whatman® filter paper No 41 and the amount of gatifloxacin dissolved in each phase, was determined spectrophotometrically at 286 nm against reagent blank prepared in the same manner on a UV-visible spectrophotometer (Shimadzu 1700).

Solubility studies

The solubility of the gatifloxacin drug sample was determined in distilled water, buffer solutions of pH 1.2, 2.0, 4.0, 6.0, 7.4, 1% Tween 80, disodium edetate, and simulated lacrimal fluid. An excess amount of drug was placed into suitable screw-cap vials containing 10 ml of each solvent, as mentioned above. The vials were properly sealed and vortexed for 10 min. They were placed on the orbital flask shaker (Khera Instruments Pvt. Ltd. India) at room temperature for 24 h. Then the samples were filtered, suitably diluted, and analyzed spectrophotometrically using a UV-visible spectrophotometer (Shimadzu 1700) at 286 nm.

Preparation of formulations

Boric acid and disodium edetate were dissolved in distilled water. *Gellan gum* and HPMC were then dissolved in the above solution. The required quantity of gatifloxacin to give a final drug concentration of 0.3% w/v was added to the polymeric solution and stirred until dissolved and then phenylmercuric nitrate was added to it as a preservative. The formulations were filled amber colored glass vials, closed with rubber closures and sealed with aluminum caps. The formulations, in their final pack, were terminally sterilized by autoclaving at 121 °C temperature, 15 psi pressure for 15 min. The sterilized formulations were stored in a refrigerator until further use.

Gelation studies

The gelation studies were carried out in cylindrical tubes, which were filled with 5 ml of simulated lacrimal fluid (SLF). 50 µl of the formulation containing ponceau red dye was added with a conventional dropper to a tube containing SLF and was then visually assessed for the gel formed and time for gelation as well as time is taken for the gel formed to dissolve.

Evaluation of formulations

Physiochemical characterization

The clarity of the formulation was evaluated by visual observation against white and black backgrounds for presence of any particulate matter. pH of the formulations was determined by pH meter (Cyberscan 510).

Drug content uniformity

Drug content analysis of prepared *in-situ* gelling systems was carried out using a spectrophotometric method. The assay of these formulations was carried out by diluting equivalent of 100 µl of (Gatifloxacin) the formulation to 25 ml with distilled water in the sterilized volumetric flask. The absorbance was estimated spectrophotometrically at 286 nm by using a double-beam UV-visible spectrophotometer (Shimadzu 1700).

Viscosity and rheology

The viscosity of the formulation was determined by Brookfield Viscometer (LVT model) [13]. To assess the gelation of formulation on instillation and mixing with lacrimal fluid, the viscosity measurements were also taken after diluting the formulation with the simulated lacrimal fluid (SLF). SLF comprised of 0.670 g sodium chloride, 0.200 g sodium bicarbonate and 0.008 g calcium chloride dihydrate and distilled water q. s to 100 g, which simulated the cation content of lacrimal fluid [19, 31]. Viscosity of the sample solution was measured over a range of 0.3 to 30 rpm speed. The hierarchy of speed was reversed from 30 to 0.3 rpm. The average of the two dial readings was used to calculate the viscosity.

Gel consistency test

The firmness, consistency and cohesiveness of hydrogels are assessed using a texture analyzer, which mainly indicates the flow behavior of sol so the formulation can be easily administered *in vivo*. Higher values of adhesiveness of gels are needed to maintain intimate contact with surfaces like tissues [32]. Gel consistency was studied using Texture Analyzer TA-XT Plus (Stable Microsystems). Gelation of the formulation was induced by adding SLF to it. A test was carried out in a standard back extrusion rig using 50 mm (diameter) container filled approximately up to 75% with the formulation. An extrusion disc of 45 mm diameter was positioned centrally over the sample container. The probe used was calibrated for the force and distance measurement before use [33]. Positive area in the graph represents consistency and positive force represents firmness of sample. Negative area represents an index of viscosity and negative force represents cohesiveness.

Isotonicity evaluation

Isotonicity, which is an important characteristic of the ophthalmic formulations, had to be maintained to prevent tissue damage and irritation to the eye. Smear of resuspended RBCs with gatifloxacin *in-situ* gel formulation was prepared and observed under the polarizing microscope (Leica) at 45x magnification [34, 35]. The same procedure was followed for the marketed gatifloxacin eye drops (Gatiquin™), isotonic solution (negative control) as well as hypertonic and hypotonic solution (positive controls). Size and shape of the RBCs with developed gatifloxacin *in-situ* gel formulation was compared to that with marketed gatifloxacin eye drops (Gatiquin™) as well as with the positive and negative controls.

***In vitro* drug release study**

The *in vitro* drug release of the gatifloxacin *in-situ* gel formulation was estimated using modified USP dissolution apparatus-1 (Electrolab). Whatman® filter paper No 41 was placed in the USP basket. It was then wetted by dipping in a solution of simulated lacrimal fluid for one minute to ensure the intimate contact of the release medium with the formulation. Then 100 µl of the formulation was applied to it. Fifty ml of simulated lacrimal fluid was filled in a beaker and the basket was rotated over its surface. A3-3 ml aliquots of samples were withdrawn at regular time intervals and replaced with an equal volume of fresh simulated lacrimal fluid. The samples were analyzed spectro-photometrically for gatifloxacin content using double beam UV-visible spectrophotometer (Shimadzu 1700) at 286 nm.

Kinetic modeling

To study the release kinetics, data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time to obtain a Korsmeyer Peppas plot. As the exponent n was 0.493 and y = 0.493x+0.734 R^2 = 0.989, it represented non fickian diffusion [36, 37].

***In vitro* trans corneal permeation study**

Whole eyeballs of goat were procured from slaughterhouse and safely transported to laboratory in cold condition. The corneas were carefully removed along with 5-6 mm of surrounding sclera and washed with cold saline solution. The washed corneas were stored in cold, freshly prepared solution simulated lacrimal fluid. Cornea was tied to one end of the hollow cylindrical glass tube and then its surface was wet by applying 14 µl of simulated lacrimal fluid to simulate the physiological condition of the eyes. [19] 100 µl of developed gatifloxacin *in-situ* gel formulation was spread over the corneal surface. The cylinder was then dipped into 50 ml of SLF maintained at 37±0.5℃ so as to keep the corneal surface in contact with SLF stirred by rotation at 50 RPM. A 3-3 ml aliquots of fluid were withdrawn at fixed time intervals over a period of 7 h and was replaced with an equal volume of fresh SLF maintained at the same temperature. Aliquots of withdrawn samples were analyzed for drug content using a double beam UV-visible spectrophotometer (Shimadzu 1700) at 286 nm.

***In vitro* antimicrobial efficacy study**

In vitro antimicrobial efficacy study was carried out to ascertain the biological activity of ophthalmic sol-to-gel system against microorganisms. It was performed by an agar diffusion test using a cup-plate technique [19]. As gatifloxacin shows activity for both gram-positive and gram-negative microorganisms, *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC27853) were used for the study. Strains were available at ISF Moga, which were procured from MTCC Chandigarh and are used for the present study. Developed gatifloxacin *in-situ* gel formulation, marketed eye drops of gatifloxacin (Gatiquin™) and placebo was poured in cups bored into the sterile nutrient agar previously seeded with *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923). After allowing diffusion of solution for 2 h, the agar plates were incubated at 37±0.5 ℃ for 24 h. The entire operation except the incubation was carried out under laminar airflow unit.

Ocular irritation study (HET-CAM test)

In developing a novel ophthalmic delivery system, an injury to the eye was taken into consideration. Since, eye being a sensitive, most delicate and yet most valuable of the sense organs, the injuries to the Cornea, Conjunctiva, and Iris were measured according to HET-CAM test (Hen's Egg Test-Chorio Allantoic Membrane). The potential ocular irritancy of a test substance was measured by its ability to induce toxicity in the *Chorio Allantoic Membrane* of a chick embryo [15, 23]. Fertilized hen's eggs weighing between 50-60 g were procured from a poultry farm. The eggs were then candled to discard the defective ones and were then incubated in a humidified incubator at 37 ℃ temperature and 75±5% RH. The trays containing eggs were rotated manually in a gentle manner every hour. After ninth days, a window (2x2 cm) was cut on the pointed end of eggs through which 0.2 ml of gatifloxacin *in-situ* gel formulation was instilled. A 0.9% NaCl solution was used as a negative control because it is reported to be practically non-irritant being isotonic and physiologically compatible and 1% NaOH as a positive control in the present study. After instillation of the formulations, the scores were recorded [38, 39].

RESULTS AND DISCUSSION

Preformulation studies

Determination of λ_{max} By UV spectrophotometer

The characterization of the gatifloxacin drug sample was done using spectrophotometric analysis. Gatifloxacin solution was scanned on a double-beam UV-visible spectrophotometer (Shimadzu 1700) between wavelength range of 200 nm to 400 nm as shown in fig. 1 and exhibited λ_{max} 286 nm which matches with the spectrum of gatifloxacin as reported in the literature [24, 25]. So, this wavelength was used for the analysis of gatifloxacin solutions throughout the studies.

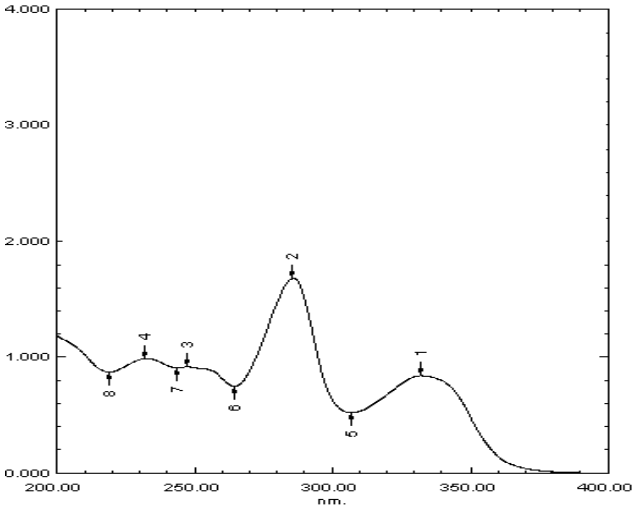

Fig. 1: UV spectrum of gatifloxacin drug sample in distilled water

Infrared spectral analysis of gatifloxacin drug sample

Infrared spectrum of the gatifloxacin drug sample showed absorption bands for carbonyl group of quinone (1631 cm^{-1}), methoxy group (2802 cm^{-1}), unsaturation (3010 and 3076 cm^{-1}), secondary amide (3404 cm^{-1}), aromatic tertiary amine C-N stretch (1363 cm^{-1}), and Ar-F (1209 cm^{-1}) as represented in fig. 2. It confirms the structure of drug as IUPAC name represents 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(methylpiperazin-1-yl)-4-oxo-3-qunolinecarboxylic acid sesquihydrate [26]. Additional fluorine atom is present at position 6 of the quinolone core, which affects the pharmacokinetic profile of fluoroquinolones in polar environments and improves their bioavailability and antibacterial activity.

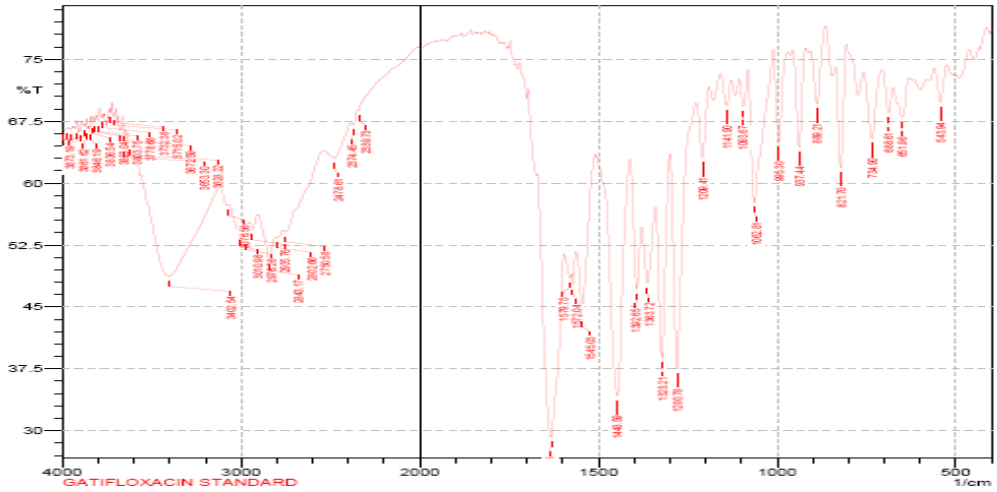

Fig. 2: FTIR spectrum of gatifloxacin drug sample

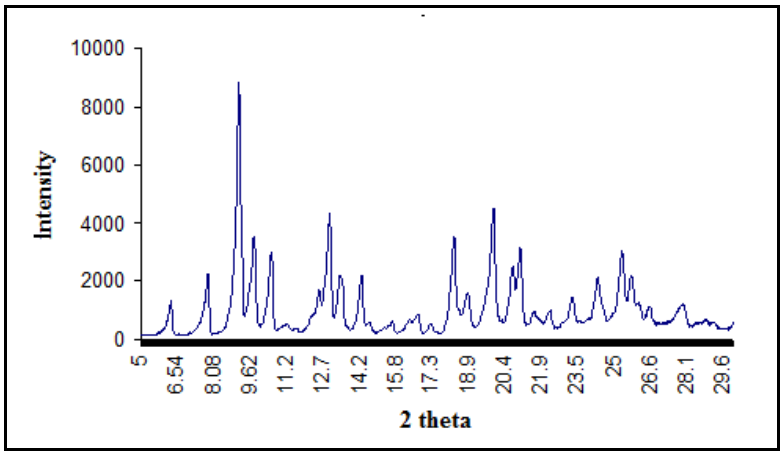

Fig. 3: X-ray diffractogram of gatifloxacin drug sample

Melting point determination

The melting point range of the gatifloxacin sample was founded around 180-183 °C, which is very close to the theoretical melting point i.e., 182-185 °C indicating gatifloxacin with high purity [26].

X-ray diffraction study of gatifloxacin drug sample

The X-ray diffractograms in fig. 3 represents the values of scattering angles (2θ), the inter planer *d*-spacings (*d*-value), and the relative intensities (I/Io), which were automatically obtained on a digital printer and confirmed the crystalline structure of gatifloxacin drug sample [28]. It was inferred that the procured drug sample was of pure gatifloxacin sesquihydrate.

Analytical method development

Preparation of calibration curve of gatifloxacin in distilled water

Appropriate dilutions were made in distilled water in the concentration range of 2-14 µg/ml and were analyzed spectrophotometrically at 286 nm against a blank prepared in the same manner. The absorbance data for different concentrations were subjected to regression analysis. The observations are graphically represented in fig. 4. Equation of the line and R-square value was found to be. Conc. = 0.087 * A, R=0.999. The linearity of the calibration curves showed that Beer Lambert's law was obeyed in the concentration range of 4-14 µg/ml at λ_{max} 286 nm

Preparation of calibration curve of gatifloxacin in simulated lacrimal fluid

Calibration curves of gatifloxacin in the simulated lacrimal fluid as represented in fig. 5 was prepared using a double beam UV-visible spectrophotometer (Shimadzu 1700). The linearity of the calibration curves showed that Beer Lambert's law was obeyed in the concentration range of 2-14 µg/ml at λ_{max} 286 nm. Equation of the line and R-square value was found to be. Conc. = 0.097 * A, R=0.999. The linearity of the calibration curves showed that Beer Lambert's law was obeyed in the concentration range of 4-14 µg/ml at λ_{max} 286 nm.

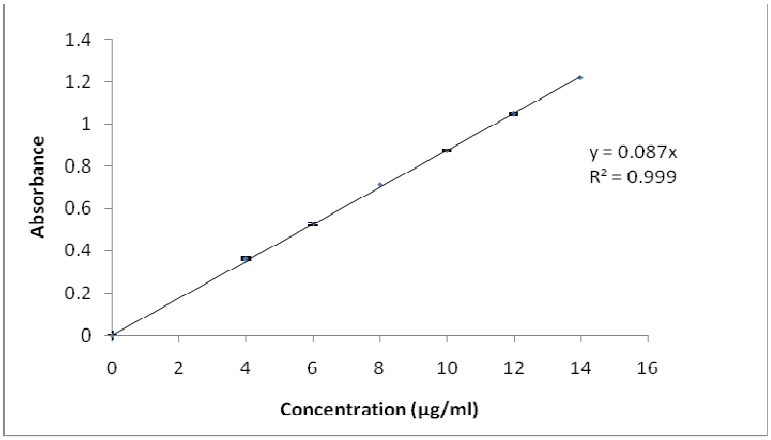

Fig. 4: Calibration curve of gatifloxacin in distilled water at 286 nm (mean±SD, n=3)

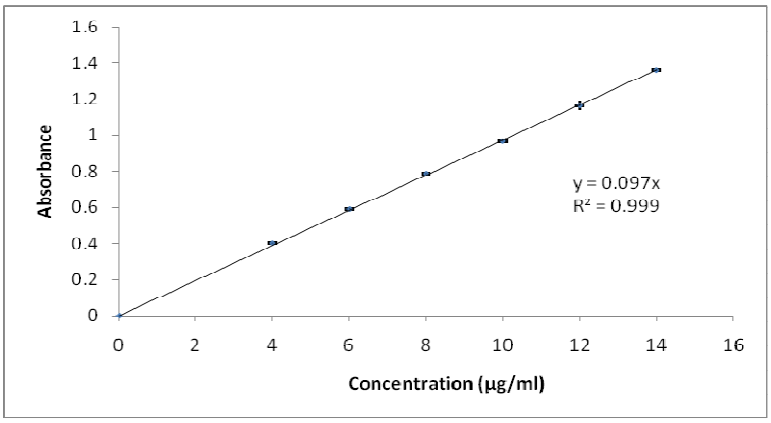

Fig. 5: Calibration curve of gatifloxacin in the simulated lacrimal fluid at 286 nm (mean±SD, n=3)

Table 1: Solubility of gatifloxacin drug sample in different solvent media

S. No.	Solvent	Amount of drug dissolved (mg/ml)	Inference [30]
1	Distilled water	2.56±0.7	Slightly soluble
2	Hydrochloric acid buffer (pH 1.2)	64.3±1.2	Soluble
3	Hydrochloric acid buffer (pH 2.0)	56.8±0.9	Soluble
4	Acetate buffer (pH 4.0)	44.4±0.9	Soluble
5	Phosphate buffer (pH 6.0)	7.6±0.5	Slightly soluble
6	Phosphate buffer (pH 7.4)	3.73±0.1	Slightly soluble
7.	Simulated lacrimal fluid	3.81±0.2	Slightly soluble

The values are expressed as mean±SD= Standard Deviation from the mean, n=3

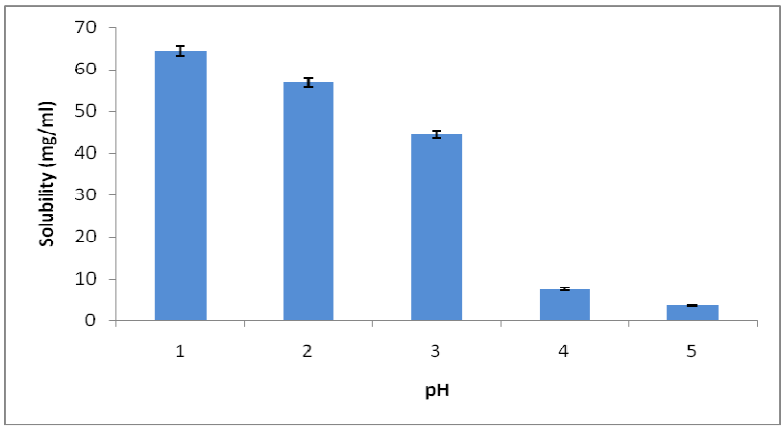

Fig. 6: pH dependent solubility profile of gatifloxacin drug sample (mean±SD, n=3)

Partition coefficient (Log P) determination

The Partition coefficient was calculated as the ratio of concentration of the drug in octanol to the concentration of the drug in water and then its logarithm was taken. The octanol-water partition coefficient (log P) of drug sample was found to be 0.59, which was similar to the calculated value from its structure, i.e., ClogP 0.6 and also matches with that reported in the literature [29].

Solubility studies

It was also found that gatifloxacin having a crystalline structure exhibit pH-dependent solubility with an aqueous solubility of 40-60 mg/ml at a pH range of 2 to 4 as represented in table 1 and fig. 6.

Preparation and optimization of formulation

Composition of *in-situ* gelling formulations is represented in table 2. *Gellan gum* was selected as an ion activated phase transition polymer for gel formation *in-situ*. Different concentrations of *gellan gum*, i.e., 0.1-0.7% were evaluated for the gelling property in physiological conditions. Out of which, only *gellan gum* solution of 0.6% exhibited desirable flow characteristics and resulted in instantaneous gelation in simulated lacrimal fluid, which was retained for an extended period of time as shown in table 3. Buffer solutions were earlier tried to impart the buffering capacity in the formulation, but that was found to result into gel formation due to probable interaction between ions and *gellan gum*. As isotonicity is a desirable attribute of an ophthalmic formulation, sodium chloride and boric acid were studied as an isotonicity adjusting agents. Since, gel formation was observed in the formulation containing sodium chloride hence boric acid was selected as an isotonicity adjusting agent. Phenyl mercuric nitrate was used as a preservative in the formulation. A 0.05% of disodium edetate was also added to enhance the solubility of gatifloxacin in water and prevent its crystallization in freeze-thaw conditions. HPMC K100M was incorporated as a release retardant in the formulation. The formulations, in their final pack were terminally sterilized by autoclaving at 121 °C temperature, 15 psi pressure for 15 min. The sterilized formulations were stored in a refrigerator until further use. Composition of formulations is mentioned as below in table 2.

Table 2: Composition of prepared *in-situ* gelling formulations

Ingredients	Amount (g)				
	F 1	F 2	F 3	F 4	F 5
Gatifloxacin	0.3	0.3	0.3	0.3	0.3
Gellan gum	0.6	0.6	0.6	0.6	0.6
Boric acid	1.68	1.68	1.68	1.68	1.68
Phenylmercuric nitrate	0.002	0.002	0.002	0.002	0.002
Disodium edetate	0.05	0.05	0.05	0.05	0.05
HPMC K100M	-	0.3	0.4	0.5	0.6
Distilled water	100	100	100	100	100

Gelation studies

Different concentrations of *gellan gum*, i.e., 0.1-0.7% were evaluated for the gelling property in physiological conditions. Out of which, only *gellan gum* solution of 0.6% exhibited desirable flow characteristics and resulted in instantaneous gelation in the simulated lacrimal fluid, which was retained for an extended period of time. The grading of gelling capacity is based up on the time taken to form the gel and time taken to dissolve the gel in simulated tear fluid. Formulations with good gelling capacity exhibit that gelation remains for longer periods once it occurs.

Table 3: Gel formation of *Gellan Gum* with simulated lacrimal fluid (SLF)

Concentration of gellan gum	Gelling property in SLF
0.1%	no gelation
0.2%	no gelation
0.3%	immediate gelation for few minutes
0.4%	immediate gelation for few minutes
0.5%	immediate gelation for few minutes
0.6%	immediate gelation which last longer
0.7%	immediate gelation which last longer

Table 4: Drug content uniformity

Formulation code	Drug content (%±SD)
F 1	99.3±0.45
F 2	97.8±0.11
F 3	98.7±0.75
F 4	96.0±0.60
F 5	97.1±0.05

The values are expressed as mean±SD Standard Deviation from the mean, n=3

Evaluation of formulations

Physiochemical characterization

The designed formulation was found to be clear by visual examination against white and black backgrounds. The pH of the formulation was determined to be between 6.2-6.3 by using pH meter (Cyberscan® 510). Formulations resulted in gel formation in SLF, clearly indicating phase transition behavior in the physiological conditions of the eye.

Drug content uniformity

Drug content uniformity was found to be between 98.7±0.75 to 99.6±0.84 % (Shimadzu 1700) at 286 nm as shown in table 4.

Viscosity and rheology

Viscosity of the formulation was determined by Brookfield viscometer (LVT). The formulation exhibited a viscosity of 55 cps in solution form and 325 cps in gel form at 12 rpm. There were 6 folds

increase in the viscosity of gel as compared to its sol form. It exhibited pseudoplastic behavior as there was shear thinning with the increase of angular velocity.

Gel consistency test

The developed formulation was further subjected to gel consistency studies on Texture Analyzer (TA-XT Plus). The consistency, firmness and cohesiveness of *in-situ* gelling system are assessed by using this device. Higher values of adhesiveness of gels are needed to maintain intimate contact with the mucus surface. Gel formed in simulated lacrimal fluid exhibited a consistency of 216.59 gm. sec, firmness 28.344 gm, index of viscosity 37.465 gm. sec, and cohesiveness-17.896 gm as shown in table 5 and fig. 7. The purpose of texture analyses was to provide information about the mechanical properties of samples. These properties can be directly correlated with sensory parameters *in vivo* and, therefore, are valuable in the development of products with desirable attributes that contribute to patient acceptability and compliance.

Table 5: Results of gel consistency test of developed gatifloxacin *in-situ* gel formulation

Area F-T 1:2 gm. sec	Area F-T 3:4 gm. sec	Force 1 gm	Force 2 gm
216.592	-37.465	28.344	-17.896

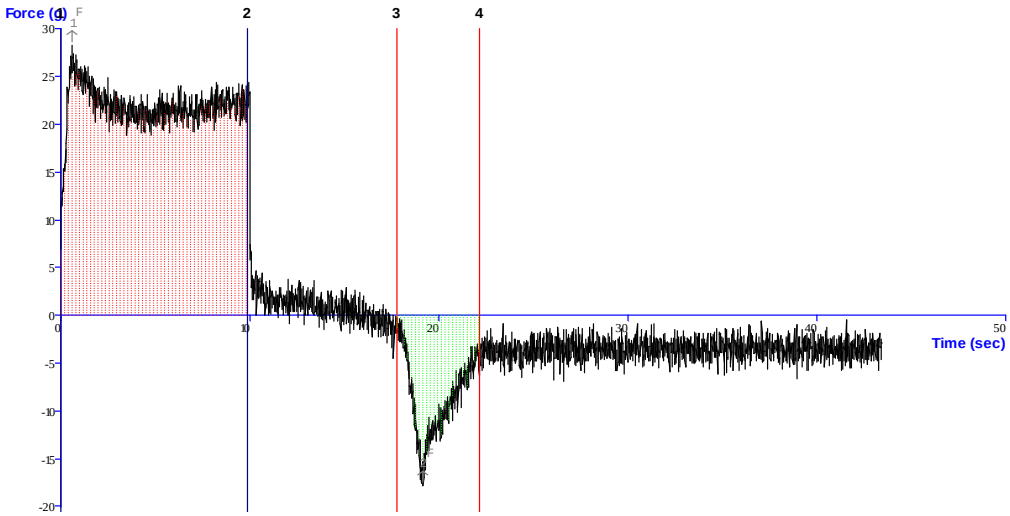

Fig. 7: Gel consistency test of developed gatifloxacin *in-situ* gel formulation

Isotonicity evaluation

Gatifloxacin *in-situ* gel formulation was found to be isotonic, as it exhibited no change in the size and shape of RBCs. The formulation was evaluated for the isotonicity and was also compared with the marketed eye drops, isotonic solution (negative control), hypertonic and hypotonic solution (positive controls). There was no change in the shape of the RBCs after the addition of developed gatifloxacin *in-situ* gel formulation, while hypertonic solution resulted in shrinkage of the cells and hypotonic solution caused the bursting of the cells.

Hence, it was confirmed that the formulation was isotonic to the eyes. It was also compared with that of marketed gatifloxacin eye drops (Gatiquin™) as shown in fig. 8.

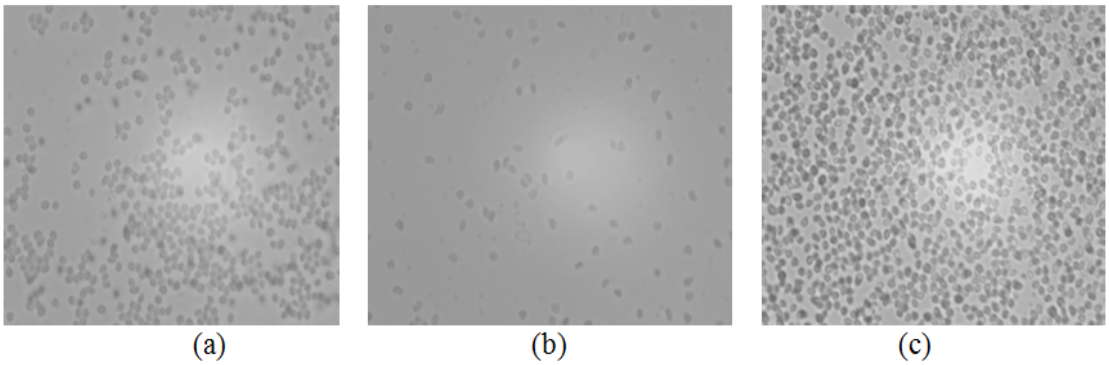

Fig. 8: (a) RBCs with developed *in-situ* gel formulation of gatifloxacin F3, (b) RBCs with marketed gatifloxacin eye drops (Gatiquin™), (c) RBCs with isotonic

***In vitro* drug release study**

Combination of 0.6% *gellan gum* and 0.4% HPMC was selected, as it had satisfactory attributes of *in-situ* gelling property, flow characteristics and prolonged *in vitro* release over the duration of 8 h with 90.6% release as shown in fig. 9.

Kinetic modeling

Data obtained from *in vitro* drug release studies were plotted as log cumulative percentage drug release versus log time, which is Korsmeyer Peppas plot [36, 37] as shown in fig. 10. As the exponent n was 0.493, it it followed the anomalous transport. The drug release pattern obtained for

the gelled samples as represented in fig. 9 and fig. 10 are characteristic for hydrophilic matrices. It is rapid in the beginning and proceeds at a rate that declined with time. When the formulation comes in contact with the simulated lacrimal fluid and gelation occurs, a pre-hydrated matrix is formed in which hydration and water penetration no longer limit drug release, leading to an apparent diffusion-controlled release. As *gellan gum* is a biodegradable polymer, it followed diffusion and matrix erosion of HPMC occurred due to hydrolytic cleavage of polymer chains and the result of kinetic data revealed that gatifloxacin followed zero-order release kinetics independent of time.

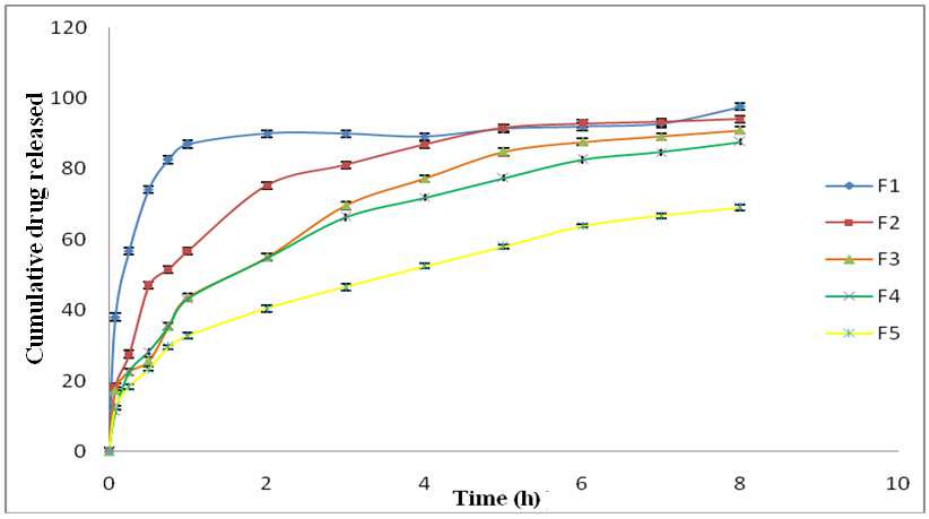

Fig. 9: *In vitro* drug release profile of gatifloxacin *in-situ* gel formulation in simulated lacrimal fluid (SLF) (mean±SD, n=6)

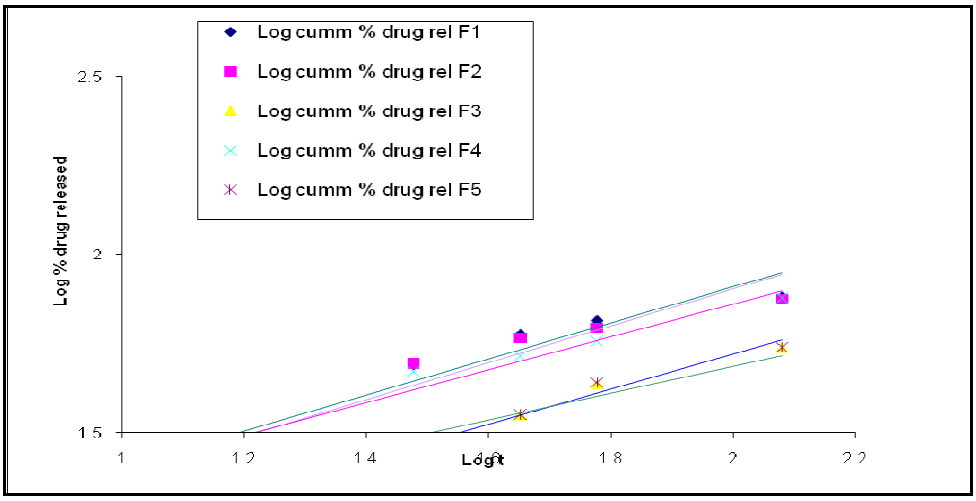

Fig. 10: Representation of korsmeyer peppas plot (mean±SD, n=6)

In vitro trans corneal permeation study

Tran's corneal drug permeation study for gatifloxacin *in-situ* gel formulation was performed in the SLF (pH 7.4 and 37±0.5 °C) which exhibited the delayed release of drug from the polymeric matrix. The *in vitro* trans corneal permeation study across excised goat's cornea exhibited 74.8% drug permeability in 7 h with an apparent permeability coefficient of 8.25 x 10^{-5} cm/sec. Prolonged precorneal retention in the conjunctival sac and slow drug release from the formulation could impart improved *in vivo* ocular bioavailability.

In vitro antimicrobial efficacy study

Diameter of the zone of inhibition observed with the developed formulation was higher as shown in fig. 11 than that of marketed preparation Gatiquin ™. Overall values of the diameter of the zone of inhibition against *P. aeruginosa* (6.6 cm) were higher than that of *S. aureus* (4.3 cm). The highest values obtained for the developed formulation in comparison to the marketed eye drops, which was 5.3 cm for *P. aeruginosa* and 4 cm for *S. aureus* could be attributed to slow and prolonged diffusion of the drug from the polymeric solutions.

Ocular irritation study (HET-CAM test)

Developed formulation was also found to be non-irritant to eyes. It was tested for irritation on the Chorio Allantoic Membrane of the chick embryo, which is a complete tissue, including veins, arteries and capillaries and responds to injury with a complete inflammatory process, a process similar to that induced in the conjunctival tissue of rabbit eyes [15, 23]. Each CAM was observed for 5 min after instillation for hemorrhage, coagulation and vessel lysis [40, 41]. Gatifloxacin *in-situ* gel formulation was found to be non-irritant to eyes exhibiting mean score of zero in HET-CAM test for ocular irritancy for 5 min as shown in table 6. Since the immune response generated by chorioallantoic membrane of chicken simulates the ocular immune response of the human eye, the developed formulation can be presumed to be non-irritant to the eyes.

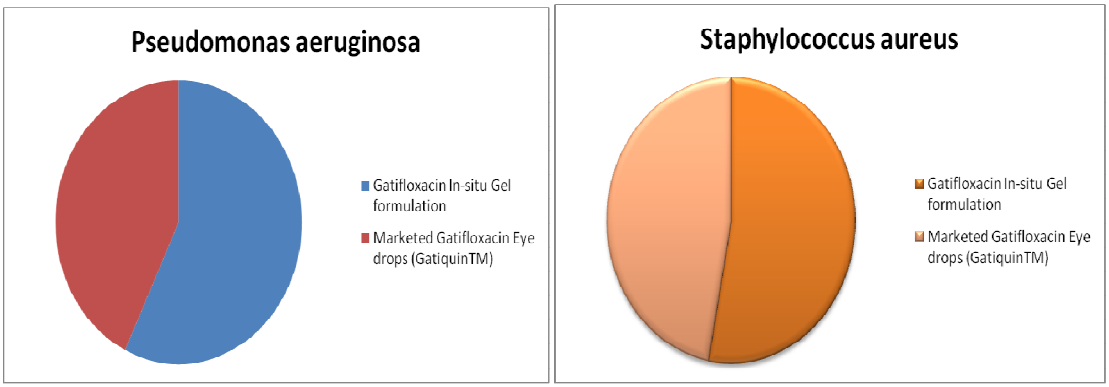

Fig. 11: Zone of inhibition exhibited by the developed gatifloxacin *in-situ* gel formulation F3 and marketed eye drop (Gatiquin™)

Table 6: Observations for HET-CAM test

Test substance	Score	Inference
0.9% NaCl	0	Non-irritant
Developed Formulation	0	Non-irritant
1 % NaOH	14.27	Severe irritant

CONCLUSION

On the basis of observation made and results obtained, it can be concluded that the developed gatifloxacin *in-situ* gel formulation can overcome the drawbacks of the conventional ocular dosage forms. The developed formulation provided efficient therapy through the prolonged drug release of the drug over an 8 h period *in vitro*. A formulation could be easily removed from the package, presented a good spreadability on the corneal surface and adhere to the mucous layer without disintegrating, in order to prolong retention time. It exhibited better antimicrobial efficacy when compared with the marketed eye drops. The formulation was isotonic and devoid of any irritant effect to the eyes. The ease of administration, along with its ability to provide sustained release could result in a decrease in the frequency of administration thus enhancing patient compliance.

ABBREVIATION

SLF-Simulated Lacrimal Fluid, HET-CAM-Hens Egg test-Chorio Allantoic Membrane, HPMC-Hydroxy Propyl Methyl Cellulose, UV-Ultra Violet, RBC-Red Blood Cells, MTCC-Microbial Type Culture Collection and Gene Bank

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors reported no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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