Research Article

Formulation and Evaluation of A Nanoparticle Laden In Situ Gel System for Enhancing the Ocular Delivery of Ciprofloxacin

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ABSTRACT

The human eye can be a tricky issue for topical administration of the drugs due to its unique anatomical arrangements of surface tissue and corneal impermeability. Topical instillation of drugs in the form of eye drops is the major and well-accepted route of administration for the treatment of varied eye disorders. Conventional ophthalmic drug delivery systems often lead to poor bioavailability and thus reduced therapeutic response. Several new preparations are developed to enhance the contact time of the medicament on the surface of the eye. Successful results have been obtained in the form of inserts and collagen shields. However, these preparations have also some disadvantages, such as poor patient compliance, particularly in the case of elderly patients. These problems could be solved by using nanoparticles laden *in situ* gel-forming systems that exhibit phase transition from solution to gel. These nanoparticle *in situ* gel systems may be formulated as eye drops suitable for administration through instillation into the eye, which upon exposure to the eye, stimulated by various ocular physiological factors, converts to the gel phase. The advantage of those formulations is that unlike inserts and films they do not require complicated equipment for manufacture and that they are scalable without any difficulty. The objective of the present study was to prepare a pH-dependent nanoparticle-laden *in situ* gel system for Ciprofloxacin, to prolong the release of the drug into the ocular compartment. No incompatibility was found between the drug and the excipients. Nanoparticles were developed using the nanoprecipitation technique. Eudragit RL 100 was used as the polymer. While the *in situ* gel solution was formulated using chitosan as polymer. The Ciprofloxacin nanoparticles were measured for particle size and the average particle size was ranged from 295.3-458.7 nm. Entrapment efficiency ranged from 13.83% to 6.29%. Nanoparticle-laden *in situ* gels had the pH of the formulations ranged from 6.01 to 6.02. Viscosity at ocular pH 7.2 ranged from 75.0 cps to 520.4 cps. The nanoparticle formulation was found to follow first-order release pattern ($r^2 = 0.985$) while the final nanoparticle-laden *in situ* gel formulation followed Higuchi or diffusion-controlled release pattern ($r^2 = 0.982$). 40.5% of the drug was able to permeate after 6 hours of *in vitro* permeation study. The accelerated stability study showed that the formulation was stable after a month.

Keywords: *In situ* gelation; Ocular drug delivery; pH-sensitive gels; nanoparticles.

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INTRODUCTION

Ciprofloxacin is said to be the most potent of the 1st generation fluoroquinolones. It is found to be active against a broad spectrum of bacteria while most effective against the aerobic gram-negative bacilli, especially the Enterobacteriaceae and *Neisseria.* About >0.1 µg/ml is the MIC of ciprofloxacin against these bacteria while the comparatively higher concentration can inhibit the gram-positive bacteria. It produces its action through inhibition of bacterial DNA gyrase and topoisomerase IV [28,29,31].

Much attention has been placed in recent decades on the development of regulated and long-term medication delivery systems. The peculiar shape of the eye inhibits pharmaceutical molecules from entering the zone of action. There are two types of drug delivery to the eye: anterior and posterior [1]. Eye drops, suspensions, and ointments are not appropriate treatments for vision-threatening ocular disorders. Polymeric drug delivery systems have been extensively researched. There is a lot of interest in the development of in situ gel systems [2]. The delivery system consists of ocular in situ gels, which can be administered as eye drops but instantly gel upon contact with the eye. In situ-forming hydrogels are liquid when injected and undergo a phase transition in the ocular cul-de-sac to produce a viscoelastic gel that responds to environmental changes [3,4]. Temperature changes, electric fields, light, pressure, sound, and magnetic fields are examples of physical stimuli; pH variations and ion activation from biological fluids are examples of chemical stimuli, and glucose level change is an example of biological or metabolic stimuli [5,6]. In the ophthalmic drug delivery system, only pH, ion activation, and temperature stimuli are used.

In-place drug delivery systems offer advantages such as reduced administration frequency and improved patient compliance and comfort. For achieving effective plasma drug concentration, an in situ gel formulation offers a novel alternative to typical delivery approaches [32,33,30]. The

in situ gelling procedure ensures accurate dosing while also increasing the medication's residence time in contact with the mucosal barrier, so addressing the difficulties associated with semisolid dosage forms. It is important to note that in situ gel is a sol that exists outside the body and changes to a gel when injected. The dosage form has received a lot of attention because it has many benefits, such as more accurate dosing [7,8]. As a result, in situ gelling technology eliminates the deleterious effects of pulsed dosing caused by traditional drug delivery systems. The medication administration system ensures that medications are given consistently and in a controlled manner. By increasing the pharmacological retention period, in situ gels improve ocular bioavailability [9]. Polymers that successfully cling to the corneal surface can be used to accomplish this. The dose form allows for ocular globe targeting while avoiding loss to other ocular tissues, as well as offering comfort, improved patient compliance, and increased pharmaceutical therapeutic efficiency [26,27]. When a medication achieves the minimal therapeutic concentration at the targeted site of action, a pharmacological reaction is induced. Obtaining adequate concentration at the site of action in eye medicines is a key difficulty. Tear formation, transient residence period, and non-permeability of the corneal epithelial layer all contribute to the limited bioavailability and absorption of ocular dosage forms. Other problems associated with low ocular dose form bioavailability include lachrymal protein binding, a small corneal surface, and metabolism. Standard ophthalmic solutions fail to attain the minimum therapeutic concentration due to rapid pre-corneal drug clearance, which can be minimized and overcome by adopting a sol-gel method [10,11].

In situ gel does not cause impaired eyesight or pain after administration. These are delivered as a cul-de-sac solution that transitions from sol to gel-based on physiological inputs [12,13]. The topical route of administration is used in the treatment of ocular disorders since a lower fraction of the dose can successfully cross the blood-retinal barrier. As a result, while the eye appears to be a desirable and easily accessible target organ for topical treatment, drug moiety absorption from the corneal surface is similarly restricted [14]. When polymeric solutions are subjected to the physiological temperature, pH, or ionic composition of the lachrymal fluid, the gel, resulting in drugs with a longer pre-corneal residence duration and, as a result, increased bioavailability [15]. The most often utilized natural polymers for in situ gel-based ocular medication administration are gellan gum, alginic acid, and xyloglucan. Because of the various defensive systems in the eye that protect the visual pathway from foreign particles, topical application of the formulation to the eye is a challenge [16].

MATERIALS AND METHODS

Drug: Ciprofloxacin (obtained from Albert David Limited)

Polymer: Eudragit RL100

Polymer: Polyvinyl alcohol

Polymer: Chitosan

Methodology for the preparation of ciprofloxacin nanoparticles

For the preparation of ciprofloxacin nanoparticles, the Nanoprecipitation method was used.

Nanoprecipitation method

Nanoprecipitation is quite a simple method used for encapsulation of both hydrophilic and hydrophobic drugs in nanoparticles. The strategy leads to the instantaneous formation of nanoparticles. It is simple to perform the technique, is easily scaled up, and is a one-step procedure. The method requires the addition of two solvents that are miscible with one another and leads to the spontaneous formation of nanoparticles on phase separation. From the 2 solvents ideally, the primary one (solvent) is that the one within which the polymer and also the drug dissolves but not within the second system (the non-solvent). A modified nanoprecipitation method utilizes the use of a co-solvent to either increase the entrapment efficiency of the drug in nanoparticles or a reduction in the mean particle size of the nanoparticles [17,18].

Methodology for preparation of placebo *in situ* gel base

First, the required amount of chitosan was weighed.1% acetic acid solution was prepared by dissolving 0.1 mL of glacial acetic acid in 10 mL of distilled water. Chitosan was then dissolved in 5ml of the prepared 1% acetic acid solution with continuous stirring using a magnetic stirrer. The remaining 1% acetic acid was then poured into the chitosan mixture and the solution was kept undisturbed overnight. pH adjustment to 6.0 was done using NaOH or Sodium acetate.

Methodology for the preparation of the final medicated formulation

An amount equivalent to 0.3% w/v of Ciprofloxacin Nanoparticles was added to the previously prepared in situ gel placebo base. 0.1% w/v Methyl Paraben was added to the finished formulation as a preservative. The quantity of Sodium chloride that is required to make the solution isotonic was also added. The finished Formulation was then filled into 10 mL amber-colored glass vials and terminal sterilization was performed using autoclave at a temperature of 121ºC and 15 psi pressure for 20 minutes.

Preformulation Studies

Preformulation studies are one of the most important aspects of formulation development. Preformulation studies performed on the drug were compatibility studies and solubility studies.

Drug-excipients compatibility studies

The drug-excipients compatibility study was performed using IR spectroscopy. FTIR instrument was used to obtain the IR spectra of Ciprofloxacin, its physical blend with the

other excipients, and the final optimized nanoparticle-laden *in situ* gel formulation. The FTIR spectra of Ciprofloxacin, its physical mixture with the other excipients, and the final formulation are shown on the following page.

Saturation solubility of the drug

For the determination of the saturation stability of the drug, an excess of the drug was mixed with a set volume of each of the pure solvents. The mixture was then sonicated and left undisturbed for one day so that after the blend achieves equilibrium, the surplus will be allowed to settle down. The supernatant solution was then filtered and diluted if necessary. The absorbance was determined using a UV-vis spectrometer at the corresponding λ_{max} of the drug within the solvent under study.

Evaluation Studies for Formulated Nanoparticles

Particle size determination

Particle size was evaluated employing the dynamic light scattering (DLS) technique which is also referred to as photon correlation spectroscopy (PCS). A laser is employed to illuminate the sample and therefore the fluctuations caused by the scattered are detected with the assistance of a fast photon detector at a known scattering angle θ.

Drug content determination

Drug content was determined by dissolving 1 mg of the nanoparticles in 10 mL of methanol then sonicated for about 15-30 minutes. It was then filtered using filter paper. The filtrate solution was analyzed using a UV-vis spectrophotometer at a λ_{max} of 279 nm. The obtained absorbance was put into the standard calibration curve of the drug in methanol and the amount of drug was determined using the equation of a straight line; y= *m* x + C.

Entrapment efficiency

It is determined using the following formula:

$$Entrapment\ efficiency = \frac{Amount\ of\ drug\ in\ the\ nanoparticle}{Initial\ amount\ of\ drug} \times 100$$

The amount of drug or drug determination methods has been explained in the prior section.

pH

The pH of the formulation was determined using a pH meter.

In vitro diffusion release

In vitro release study was carried out using a standard Franz diffusion cell. 50 mL of Phosphate buffer pH 7.4 was filled into the receptor chamber. The fluid in the receptor chamber was stirred constantly using a small magnetic bead and magnetic rotor at 200 rpm and the temperature was maintained at 35º C to replicate the temperature of the ocular surface. A dialysis membrane which has been activated one day before the study was used to separate the receptor and donor compartments. 1 mL of the nanoparticle solution was transferred into the donor compartment and the dialysis membrane was tied to the mouth of the donor compartment with the help of a woolen thread. The sample was withdrawn at intervals of 30mins, 1hr, 1.30 hrs, 2,3,4,5, and 6 hrs respectively. The drug content was determined by a UV-vis spectrophotometer at λ_{max} of 270nm.

Evaluation Studies for *in situ* gel

Clarity

Clarity of the formulation was assessed through visual examination of the formulation under light, before and after gelation.

Gelling capacity

A drop of the gel solution was put into a watch glass containing 2 mL of phosphate buffer pH 7.4. Visual assessment of the gelation process was done and the time taken for the gel to form and the time taken for the formed gel to dissolve was observed.

Gelation pH

The pH at which the formulation undergoes a phase transformation from solution to gel form is known as gelation pH. It is determined by increasing the pH of the solution with NaOH or Sodium acetate and observing the gel formation process visually and verifying it by checking the viscosity. The pH at which the formulation undergoes a sudden change in the viscosity is noted as the gelation pH.

Viscosity

Brookfield's viscometer was used to measure the viscosity of the formulation before and after gel formation. For the measurement of viscosity using the viscometer 50 mL of the formulation was taken.

In vitro diffusion release

In vitro release study is the same as the one during nanoparticle evaluation. It was carried out using a standard Franz diffusion cell. 50 mL of Phosphate buffer pH 7.4 was filled into the receptor chamber. The fluid in the receptor chamber was stirred constantly using a small magnetic bead and magnetic rotor at 200 rpm and the temperature was maintained at 35º C to replicate the temperature of the ocular surface. A dialysis membrane which has been activated one day before the study was used to separate the receptor and donor compartments. 1 mL of the nanoparticle-laden *in situ* gel solution was transferred into the donor compartment and the dialysis membrane was tied to the mouth of the donor compartment with the help of a woolen thread. The sample was withdrawn at intervals of 30mins, 1hr, 1.30 hrs, 2, 3, 4, 5, and 6 hrs respectively. The drug content was determined by a UV-vis spectrophotometer at λ_{max} of 270nm.

In vitro permeation study

This was done alongside the *in vitro* release study using the Franz diffusion cell. Cellulose dialysis membrane was used to simulate human cornea while pH 7.4 buffer mimicked tear fluid and simulated ocular pH environment. The sample was withdrawn at intervals of 30mins, 1hr, 1.30 hrs, 2,3,4,5, and 6 hrs respectively. The drug content was determined by a UV-vis spectrophotometer at λ_{max} of 270nm. From this cumulative percentage of the drug permeated from the receptor, the chamber was calculated.

Accelerated stability study

The finished formulation was stored for about a month and an FT-IR study of the stored formulation was done after a month. The IR spectrum was checked for any significant changes in the IR peaks for the assessment of the stability of the formulation.

RESULTS AND DISCUSSION

FTIR study

To serve the purpose of studying Drug-excipients compatibility FT-IR (Fourier-transform infrared spectroscopy) analysis was performed. FT-IR spectrum data of both pure and the final optimized nanoparticle-laden *in situ* gel formulation have been provided in this section.

Table 1: Position of IR peaks of the drug, its physical blend, and the formulation

Functional Groups	Wavenumber (cm^{-1})		
	Pure Drug	The physical blend of Drug with excipients	Formulation
O-H stretch	3530.29	3529.26	3529.78
N-H stretch of piperazinyl moiety	3378.29	3378.12	3378.18
Aliphatic C-H stretch	2924.86	2922.93	2925.62
N-C stretch	2838.47	2843.94	2841.96
C=O stretch of carboxyl group	1707.06	1706.76	1707.30
C=O stretch of quinolone	1622.72	1622.85	1623.12
C-N stretch	1454.03	1452.03	1452.64
C-F stretch	1269.43	1268.71	1269.49

Composition of Formulation

Formulations were prepared with variations based on the drug and polymer ratio in the Ciprofloxacin nanoparticle formulations and varying the % w/v of chitosan in the in situ gel formulations (Table.2). Nanoparticle formulations were white-yellowish semi-solid in appearance while the in situ formulations were slightly turbid solutions.

Table 2: Composition of Ciprofloxacin Nanoparticles laden in situ gel formulation

Ingredients	Formulation 2A (w/v)	Formulation 2B (w/v)	Formulation 2C (w/v)
Ciprofloxacin nanoparticles	0.3%	0.3%	0.3%
Chitosan	0.25%	0.5%	1%
Glacial acetic acid	1% v/v	1% v/v	1% v/v
NaCl	0.45%	0.45%	0.45%
Methyl Paraben	0.1%	0.1%	0.1%
Water (q.s.)	100%	100%	100%

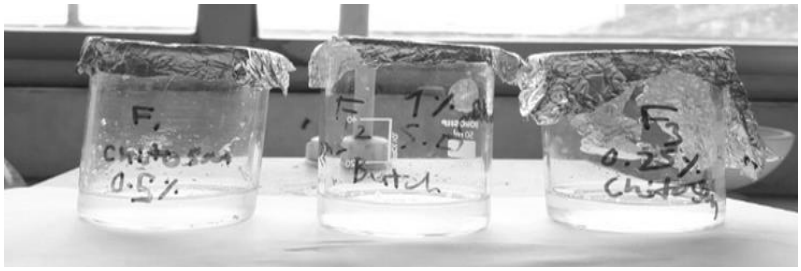

Figure 1: Ciprofloxacin nanoparticle laded in situ gel formulations

Evaluation of Ciprofloxacin nanoparticles

Particle size distribution

Unimodal distribution of particle size was observed in the analysis of particle size. Particles of size ranging from 295.3-458.7 nm were found in the formulation 1A. Maximum numbers of particles were found within the size range of 342-396.1 nm.

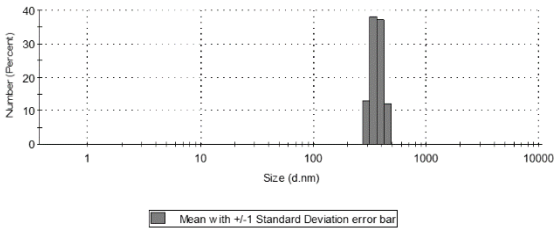

Figure 2: Particle size distribution of formulation 1A (Histogram) representation)

Drug Content determination

1 mg of the nanoparticle was weighed. The weighed amount was transferred into a 10 mL volumetric flask and volume was made up with methanol. The system was then sonicated for about 15-30 minutes. The resultant solution was filtered using Whatman filter paper. The filtrate solution was analysed using a UV-vis spectrophotometer at a λ_{max} of 279 nm. The obtained absorbance was put into the standard calibration curve of the drug in methanol and the amount of drug was determined using the equation of a straight line; y= *m* x + C.

Calculation (Formulation 1A, drug: polymer = 1:10)

1 mg of nanoparticles dissolved in 10 mL methanol.

Absorbance = 0.305 at λ_{max} 279 nm (via UV spectroscopy)

Equation of the standard curve was found to be

y = 0.138x + 0.135

Putting the absorbance value into the above equation we get Concentration = 1.2424 µg/mL

Therefore, the Drug content in 1 mg of nanoparticles = 1.2424 * 10 = 12.4248 µg

Entrapment Efficiency

It is determined using the following formula:

$$Entrapment\ efficiency = \frac{Amount\ of\ drug\ in\ the\ nanoparticle}{Initial\ amount\ of\ drug} \times 100$$

Calculation (Formulation 1A, drug: polymer = 1:10)

Initially, 10 mg of drug was taken for 100 mg of polymer

Therefore, 110 mg of formulation contained 10 mg of the drug

100 mg of formulation contained $\frac{10}{110} \times 100$ = 9.09 mg of drug 1 mg of formulation contained 0.091 mg of the drug

Hence, Initial amount of drug present = 0.091 mg or 91µg of drug

Drug Content in 1mg of nanoparticle = 12.5922 µg (calculated previously)

Therefore, Entrapment efficiency = $\frac{12.5922}{91} \times 100$ = 13.83 %

Formulation 1A with an entrapment efficiency of 13.83% was found to be the best among the three variations.

pH

pH was determined using a pH meter(5.57).

In vitro drug release study

A drug release study was performed using a Franz diffusion cell according to the procedure discussed in the methodology section and the percentage cumulative release data was calculated from the absorbance data obtained through UV spectroscopy at λ_{max} 270 nm at different intervals of time. Following shows the release profile of zero order, first order, Hixson-Crowell model and dissolution control release pattern of formulation 1A. The regression coefficients obtained from the study are tabulated below.

Table 3: Drug release kinetics of Ciprofloxacin nanoparticles (formulation 1A)

Order of Process	R^2 value
Zero-order	0.980
First order	0.985
Higuchi	0.984
Hixson-Crowell	0.982
Korsmeyer- Peppas	0.934

The values indicate that the drug release pattern shows the closest fit with the first order release profile with an r^2 value of 0.985.

Evaluation of nanoparticle laden *in situ* gel

Clarity

Clarity is determined is a visual examination of the formulation against light and dark backgrounds

Gelling capacity

Results from the evaluation of gelation capacity showed that formulation 2A remained in solution form at formulation pH didn't form a gel within the ocular pH range. Formulation 2B also remained in solution form at formulation pH but was able to form a gel around ocular pH. The gel thus formed was also stable and remained undissolved for more than 24 hours. But formulation 2C remained in gel form even at the formulation pH. Gelation pH for the formulations was found to be greater than pH 10 for formulation 2A, at pH 7.2 for formulation 2B, and approximately around pH 4 for formulation 2C.

Gelation pH

Formulation pH is the pH of the formulation before any pH adjustments while gelation pH is the pH at which the formulation undergoes solution to gel transformation.

Viscosity

The viscosity of the formulations was measure using Brookfield's viscometer (Model DV- II + pro, spindle no. 62) at 20 rpm at a temperature of 30º C.

Accelerated stability study

For the accelerated stability testing IR spectra of the formulation before and after storage up to one month were compared.

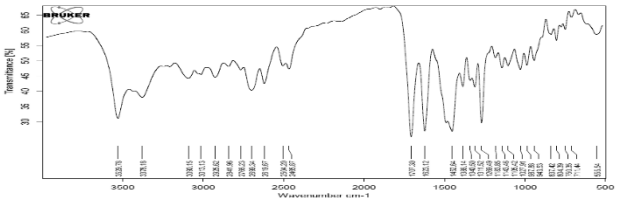

Figure 3: FT-IR spectrum of nanoparticle laden in situ gel formulation

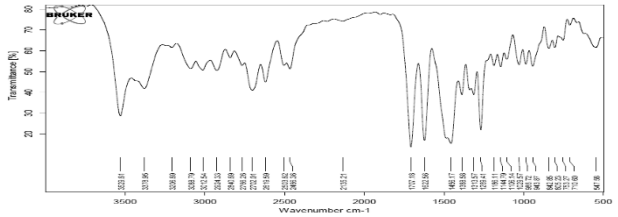

Figure 4: FT-IR spectrum of nanoparticle laden in situ gel formulation (after 1 month)

It was found that there were no significant changes in the IR peaks values from the spectrum obtained after the formulation was kept in storage as compared to the formulation before storage. This implies that the formulation remained stable during this period of storage time.

CONCLUSION

This study was based on the formulation of Ciprofloxacin-laden in situ gels to improve the ocular retention of Ciprofloxacin. Thanks to the precorneal loss factors which include rapid tear turnover, non-productive absorption, duration of transient residence within the cul-de-sac, the drug are relatively impermeable to the corneal epithelial membrane. Ciprofloxacin nanoparticles were prepared using Eudragit RL 100 as the polymer and later incorporated into in situ gel base made from chitosan, which improved permeability of the drug as well as release properties. Eudragit RL 100 is a copolymer made from methyl methacrylate, ethyl acrylate, and a small amount of methacrylic acid ester with quaternary ammonium groups. The polymer is permeable due to the presence of the ammonium groups in form of salts.

A key problem that is faced in the ophthalmic dosage forms is to achieve an optimal concentration at the target site. This is mainly because of the tear production, absorption without any effect, low residence time, and corneal epithelium impermeability. Eudragit demonstrates favorable characteristics for instance no toxicity, positive charge, and controlled release profile which makes it appropriate for ophthalmic applications.

Chitosan is a linear polycationic copolymer of β (14) linked 2-acetamide-2 deoxy-β-D- glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose obtained from deacetylation of chitin, the second most abundant natural polysaccharide. Chitin is a crucial constituent of the exoskeletons of animals. Especially crustaceans (crabs, shrimps, and lobster), mollusks, and insects [19,20]. It's also the principal fibrillar polymer within the cell wall of certain fungi. Chitosan is a weak base and has a pKa value of about 6.2-7.0 for its D-glucosamine residue. Thus, it's insoluble at neutral pH and alkaline pH values. In an acid medium, the amino groups present in the polymer are protonated. This leads to a soluble polysaccharide with a positive charge (RNH_3^+) and a high charge density [21,22,23]. Chitosan shows swelling ability in the presence of aqueous media and its polymer chains self-assemble into a physically crosslinking network when the solution pH is above 6.5. This network can't be formed when the pH is low. It is often used to formulate delivery systems that are suitable for achieving sustained release of either hydrophilic or lipophilic drugs. Hence, the release of a hydrophilic drug such as Ciprofloxacin might be enhanced using chitosan. Also, chitosan can act as a penetration enhancer and may enhance the potential of Eudragit polymer [24,25].

The present study concludes that Ciprofloxacin can be entrapped as eudragitRL 100 nanoparticles within *in situ* gel base made of chitosan polymer. The mucoadhesive properties of chitosan and its permeation enhancement action on the eudragit polymer may contribute to the increased corneal residence time of Ciprofloxacin. In the case of Nanoparticle formulation, Formulation 1A with an entrapment efficiency of 13.83% was found to be the best among the three variations. In vitro release studies were carried out using Franz diffusion cells for up to 6 hours. The first-order release pattern was found to be the best fit for formulation 1A. In the case of the nanoparticle-laden *in situ* gel formulations, formulation 2B was the best one for being able to show the solution to gel transformation within the ocular pH range with its viscosity undergoing a drastic change from 37.5 cps to 292.4 cps at ocular pH of 7.2. From the results of the *in vitro* release study, Higuchi or the diffusion-controlled release pattern was found to be the best fit for formulation 2B. In the *in vitro* permeation studies it was observed that 40.5% of the drug was able to permeate after 6 hours. The accelerated stability test showed that the formulation was stable for one month. From these results, we can conclude that we were able to formulate an *in situ* gel formulation loaded with Ciprofloxacin which was stable and was able to show prolonged drug release. This will reduce the dosing

frequency of the drug. Thus, the developed-*in situ* gel formulation is an effective alternative to conventional ophthalmic drug delivery systems.

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