

# Design, Development, and Characterization of Modified Xanthan Gum Based Novel *in situ* Gel of Ciprofloxacin Hydrochloride for Ophthalmic Drug Delivery

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## Abstract

**Aim:** Ciprofloxacin hydrochloride is a broad-spectrum a powerful fourth generation fluoroquinolone antibiotic active against both Gram-positive and Gram-negative ocular pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* and used the treatment of ocular infections. In conventional dosage forms its washout, poor retention, drainage from eyes affects its therapeutic efficiency, so there is need of novel formulation of it. The aim and objectives of this research work are to explore the applicability of our previously modified xanthan gum in formulation, optimization, and evaluation of *in situ* gel of ciprofloxacin hydrochloride for ophthalmic drug delivery. **Materials and Methods:** Fourier-transform infrared (FT-IR) and differential scanning calorimetry (DSC) study was performed to find out compatibilities between drug and polymers. To find out the impact of Conc. of Modified Xanthan Gum ... (X1) and... HPMC (X2) on dependent variables, i.e., gelation temperature and viscosity 3<sup>2</sup> factorial designs Optimization technique was used. *In situ* gel was prepared using modified xanthan gum and HPMC, etc. The prepared *in situ* gel of ciprofloxacin was evaluated for pH, gelation time, gelation temperature, viscosity, drug content, syringeability, antimicrobial potential, and *in vitro* drug release. **Results and Discussion:** FT-IR and DSC study suggests no interaction between ciprofloxacin, modified xanthan gum, and HPMC. The pH of *in situ* gel solution was found to be around  $6.6 \pm 0.2$ – $6.9 \pm 0.08$  which is an acceptable range for ophthalmic preparations. Gelation time of prepared *in situ* gel solution was found to be around  $10.6 \pm 1.2$ – $32 \pm 0.8$  s. Gelation temperature of prepared *in situ* gel batches was found to be around  $30.6 \pm 0.4$ – $35 \pm 0.8$ °C. It was found that increasing the concentration of the polymer resulted in a significant increase in viscosity. Modified xanthan gum had a more pronounced effect on the viscosity than HPMC at the studied ranges. *In vitro*, drug release studies showed a polymer concentration-dependent decrease in drug release. Formulation F8 selected as an optimized batch has maximum gelling temperature  $34.6 \pm 1.2$ °C and minimum drug release 91.59% drug release. Optimized formulation gave satisfactory results in terms of antimicrobial activity. The optimization study was successfully conducted using 3<sup>2</sup> factorial designs. **Conclusion:** Developed *in situ* gelling systems are viable alternative to conventional ophthalmic products with added benefits of sustained drug released and it will promising approach toward the treatment of various bacterial infections.

**Key words:** % Gelation temperature, antimicrobial activity, bacterial infections, ciprofloxacin, modified xanthan gum and HPMC, optimization

## INTRODUCTION

The eye is a delicate and complex organ of the body.<sup>[1]</sup> Due to the unique structure of the eye restricts the entry of drug molecules at the required site of action. The development of ocular drug delivery is one of the most interesting and challenging to scientists, students, and researcher working on it.<sup>[2]</sup> The past 50 years, ophthalmic drug delivery research has made much progress. Ophthalmic

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preparations are defined in the United States Pharmacopeia as “Sterile dosage forms, free from foreign particles, suitably compounded, and packed for instillation into the eye.”<sup>[3]</sup>

There are two types of ophthalmic products such as conventional and novel.<sup>[4]</sup> 90% of the ophthalmic market share is taken by conventional eye drops because of its to simple instillation into the eye with accuracy of doses, patient compatibility and ease of production and economical cost.<sup>[5]</sup> However, those conventional ophthalmic products are associated with certain problems such as short residence time, poor bioavailability, poor permeability, rapid precorneal drainage, lachrymal fluid dilution and tear production, and limited corneal area.<sup>[6]</sup>

In past decades, many approaches are tried to retain drug in eyes which are used such as chemical permeability enhancers, prodrugs stimuli-responsive *in situ* gel, and to increase ocular residence time, so increases ophthalmic bioavailability and permeability.<sup>[7]</sup> *In situ* gelling system is one of the promising approaches to improve the retention time of drugs on the ocular surface. These *in situ* forming systems are liquid on instillation and converted into form viscoelastic gel.<sup>[8,9]</sup> Gel dosage forms are successfully used as drug delivery systems to control drug release and protect the medicaments from a hostile environment.<sup>[10]</sup> *In situ* gel-forming drug delivery systems are the principle, capable of releasing the drug in a sustained manner maintaining relatively constant plasma profiles.<sup>[11]</sup>

Day by day popularity and acceptability of *in situ* gel system are increase in medical field because they are instilled into the eye as a solution and immediately converted into a gel when it contact with the eye.<sup>[12,13]</sup> Recently, combination of polymers used in *in situ* gel system since it reduces the probability of formulation failure and reduces the quantity of polymers required to develop ocular formulation.<sup>[14]</sup>

Appropriate and safe use of antibiotics is necessary for the treatment of ocular infections.<sup>[15]</sup> Mostly fluoroquinolones were used as drug for novel in ophthalmic insert. Success of antimicrobial therapy of an infection depends on the concentration of the antibiotic at the site of infection.<sup>[16]</sup> Last past two decades, antibiotics were studied in different *in situ* gelling systems to prolong corneal contact time and enhancing ocular bioavailability ultimately to improve patient compliance.<sup>[16,17]</sup>

Ciprofloxacin belongs to the family of a powerful fourth generation fluoroquinolone antibiotic, useful in the treatment of infections of outer eye such as bacterial conjunctivitis and keratitis caused by ocular pathogens. It is a broad-spectrum antibiotic active against both Gram-positive and Gram-negative ocular pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*.<sup>[17]</sup> It works by inhibiting DNA gyrase, a Type II topoisomerase and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby

inhibiting cell division and also affect mammalian cell replication.

In conventional dosage forms its washout, poor retention, drainage from eyes affects its therapeutic efficiency so there is need of novel formulation of it.<sup>[18]</sup> Thus, to target the limitations of the traditional formulation including rapid exclusion, insignificant ocular bioavailability, and eye irritation, we have proposed an innovative formulation of ciprofloxacin hydrochloride based on multifactorial approaches.<sup>[8,19]</sup>

The aim and objectives of this research work are to explore applicability of our previously modified xanthan gum in formulation, optimization, and evaluation of *in situ* gel of ciprofloxacin hydrochloride for ophthalmic drug delivery.

## SUBJECTS AND METHODS

### Materials

Ciprofloxacin was obtained from Cipla Ltd., Mumbai, HPMC, glacial acetic acid pyridine, ammonia, and ethanol were obtained from S.D LAB Chemical center Mumbai India. Thionyl chloride was obtained from Pallav Chemicals and Solvents PVT Ltd. Andheri (W) Mumbai, modified xanthan gum, prepared in laboratory. All other chemicals were used of analytical reagent grade.

### Methods

Synthesis of modified xanthan gum as per our previously described method.<sup>[20]</sup>

### Fourier-transform infrared (FT-IR) study<sup>[21]</sup>

FT-IR study was performed to find our interaction between drug and polymers. The potassium bromide (KBr) disks with ciprofloxacin alone and formulation blend were prepared manually by press method. About 1 mg of drug was triturated with about 10 mg of dry KBr and then pressed into the pallet manually. Drug polymer ratio was 5:1. IR Spectra of Ciprofloxacin, Modified Xanthan Gum, HPMC, and their physical mixture were determined in the range of 400–4000/cm.

### Differential scanning calorimetry (DSC) study<sup>[22]</sup>

Possible interactions between the drug and the utilized polymer were analyzed from DSC thermograms of the pure drug ciprofloxacin and in the formulation obtained. DSC study was carried out. The drug, polymer (MXG and HPMC), as well as their physical mixture were weighted separately in aluminum pan, covered with aluminum lid and hermetically sealed using a pan press. The temperature of

pan was gradually increased from 25 to 300°C at a rate of 10°C/min in nitrogen atmosphere.

### Fabrication of ocular *in situ* gel of ciprofloxacin

The required amount of modified xanthan gum and HPMC were dissolved in cold water. The water was added to make up the volume. The solution was stirred for 2 h and within this period 0.3% W/V ciprofloxacin is added to the preparation and *in situ* gel was formed.<sup>[23]</sup>

### Experimental design<sup>[24,25]</sup>

Design of experiments technique was applied followed by response surface methodology to optimize formulation variables using latest version of Design-Expert\_12 Software. To find out the impact of Conc. of Modified Xanthan Gum ... (X1) and... HPMC (X2) on dependent variables, i.e., gelation temperature and viscosity 3<sup>2</sup> factorial designs Optimization technique was used. Experimental designed batches are shown in Tables 1 and 2.

### Characterization of optimized batches of ocular *in situ* gel formulation

#### Clarity and appearance

Determination of the physical properties of *in situ* gels before performing characterization studies was done. All preparations were subjected to visual examination for clarity, before and after gelation. This examination involves the visual assessment of formulation in suitable lighting

**Table 1:** Translation of coded values for 3<sup>2</sup> factorial experimental designs batches

Formulation code	Variable level code	
	X <sub>1</sub>	X <sub>2</sub>
F1	-1	-1
F2	-1	0
F3	-1	+1
F4	0	-1
F5	0	0
F6	0	+1
F7	+1	-1
F8	+1	0
F9	+1	+1

**Table 2:** Value codes of factorial design

Coded value	Modified xanthan gum (g)	HPMC (g)
-1	1	0.5
0	1.2	1
+1	1.5	1.5

on white and black background also it was observed for formation of turbidity or any unwanted particles dispersed in the solution.<sup>[26]</sup>

### Measurement of pH

Ten grams of the *in situ* gel were taken and pH values of all *in situ* gel formulations of ciprofloxacin were measured using digital pH meter, calibrated using standard pH buffer tablets of pH 4.0 and 7.0 at 25°C.

### Syringe ability study

All the developed formulations were tested syringe ability through 21-gauge needle.

### Drug content

Uniform distribution of active ingredients is important to achieve dose uniformity. For this study, 1 ml of the developed *in situ* gel formulation was dissolved in 100 ml phosphate buffer (pH = 7.4) after suitable dilution followed by spectrophotometrically estimation of the aliquot to determine the drug concentration.<sup>[27]</sup>

### Gelation temperature

The gelation temperature was determined by the tube inversion method. The test solution was placed in a test tube which was dipped in a water bath maintained at a temperature of 40.0 ± 1°C for 5 min. The temperature at which the test solution was converted to gel ceases to flow with no change in meniscus on tilting up to 90°, was recorded.<sup>[27]</sup>

### Gelation time

The gelation time was determined by gradually increasing the temperature of the formulations, and the time required by the formulations (containing different concentrations of the polymers) to form a stiff gel was recorded using a digital stopwatch. The gelation time was determined using an aluminum foil which was placed on a hot plate equilibrated at 35°C. A few drops of each test solution were instilled onto the pan using a Pasteur Pipette. The pan was then tilted at 90° to examine the gelation. The final gelation time is recorded when the free-flowing solutions transforms into a thick textured gel and ceases to flow (no change in meniscus) on tilting by up to 90°.<sup>[27]</sup>

### Measurement of viscosity

The viscosity measurements were carried out using Brookfield Viscometer. The developed formulations were placed in the sample beaker and kept for a 5 min. The viscosity of the

solutions was measured with respects to temperature at 10 rpm, 20 rpm, 50 rpm, and 100 rpm using Spindle L4.<sup>[17]</sup>

### **In vitro drug release study**

A Franz diffusion cell was used for the determination of *in vitro* drug release of *in situ* gel of ciprofloxacin hydrochloride for ophthalmic drug delivery. The receptor chamber (20 ml volume) was filled with PBS pH 7.4 and stirred constantly using small magnetic bar. The dialysis membrane, previously soaked overnight in a dissolution medium, was tied to one end of the donor cell of the Franz diffusion cell. The temperature set at 35°C. 1 ml of *in situ* gel solution was placed over the dialysis membrane. Aliquots, each of 1 ml starting from the 1<sup>st</sup> min, were withdrawn at an hourly interval and replaced by an equal volume of receptor medium. The withdrawn at predetermined time points for up to 12 h. The *in situ* gel was analyzed by ultraviolet (UV)-visible spectrophotometer (UV-2450, SHIMADZU) at 278 nm.<sup>[17-19]</sup>

### **Mathematical modeling and release kinetics**

The kinetics of *in situ* gel of the ciprofloxacin hydrochloride was determined using the release kinetics method of drug release into various kinetic equations: Zero-order release kinetics, first-order release kinetics, and Higuchi model. Various kinetic parameters are determined by using drug release data. The parameters “n” and time component “k,” the release rate constant and “R,” and the regression coefficient were determined by Korsmeyer-Peppas equation to understand the release mechanism.<sup>[19]</sup>

### **Antimicrobial activity procedure**

The antimicrobial activity was performed to evaluate the antimicrobial efficiency of the optimized formulation. Sterilize the Petri plate and conical flask by keeping them in hot air oven for 5–10 min. Taken 6 g of nutrient agar dissolved in 50 ml of water in conical flask boiled well, till bubbles noises in the conical flask (near about 15 min). Removed from the gas barrier and put a cotton plug at the mouth of conical flask autoclave for 15 min (conical flask with agar media) After 15 min pour 15 ml agar solution in each Petri plate and cool at room temperature. After 5 min added one drop of (*S. aureus*) bacterial suspension in each Petri plate of the center (with pipette) and spread evenly with the help of spreader wait for 2 min and now, makes holes using “T”-BORER in to the Petri plate as required.

Std ciprofloxacin= 100 mg ciprofloxacin dissolved in 100 ml of dilute acetic acid and was added into the volumetric flask and from this solution 1 ml was withdrawn and added into the 10 ml volumetric flask make up to the water.

*In situ* gel= 1 ml gel dissolved in 2 ml water and from this solution 1 ml was withdrawn and added into the 10 ml volumetric flask make up to the water.

Blank= 10 ml dilute acetic acid was added in to the 100 ml volumetric flask and make up to the water. From this solution, 1 ml was withdrawn and added into the 10 ml volumetric flask make up to the water. Added different solutions to be tested into the holes made. Keep in refrigerator for 5 min. Keep the above plates in incubator for 24 h and next day check the (diameter) reading.<sup>[15,20,24]</sup>

### **Selection of optimized formulation**

To optimize a formulation with better properties, latest version Design-Expert\_ 12 was used to target a formulation with maximum gelling temperature and minimum drug release. The maximum gelling temperature ensures enough time to administer the formulation while minimum drug release ensures the release of drug for a longer period of time.<sup>[28-30]</sup>

## **RESULTS AND DISCUSSION**

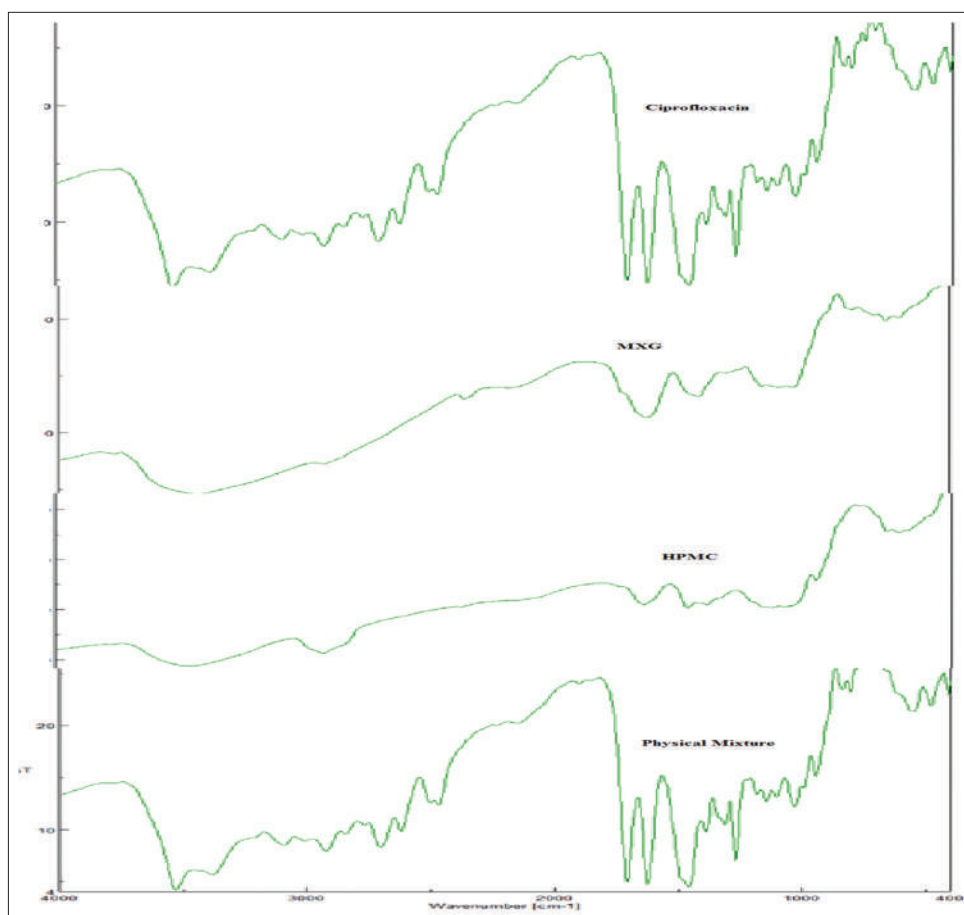
### **Drug polymer compatibility studies**

The FT-IR and DSC are used to determine the possible interactions between drug and polymers that result is presented in Figures 1 and 2.

### **FT-IR study**

The FT-IR spectrum of the pure drug ciprofloxacin and in physical mixture was compared with those of drug and polymer and matching were done to detect appearance or disappearance of the peaks. For ciprofloxacin major peaks are observed as NH starching at 3529 cm<sup>-1</sup>, aromatic CH at 3093 cm<sup>-1</sup>, aliphatic CH at 2923 cm<sup>-1</sup>, carbonyl group at 1704 cm<sup>-1</sup>, and carboxylic group at 1704 cm<sup>-1</sup> (CO), and broad peak 3000–3550 cm<sup>-1</sup> (OH). Modified xanthan gum showed peaks at 2900 cm<sup>-1</sup> of aliphatic CH, 3436 cm<sup>-1</sup> for OH, and amino group, whereas HPMC showed peaks at 2923 cm<sup>-1</sup> for aliphatic CH, and 3464 cm<sup>-1</sup> for OH group. The peaks of physical mixtures were found to be NH starching at 3500 cm<sup>-1</sup>, aromatic CH at 3050 cm<sup>-1</sup>, aliphatic CH at 2948 cm<sup>-1</sup>, carbonyl group at 1700 cm<sup>-1</sup>, and carboxylic group at 1700 cm<sup>-1</sup> (CO), and broad peak 3000–3550 cm<sup>-1</sup> (OH).

The FT-IR spectrum for physical mixture of ciprofloxacin and polymer did not show any shift in the peaks of both the drug and polymers but the intensities of some peaks are slightly decreased which might be due to dilution of the drug with the polymer compared with ciprofloxacin alone. Therefore, as per Figure 1, it can be concluded that there are no any



**Figure 1:** Fourier-transform infrared spectrum of ciprofloxacin, modified xanthan gum, HPMC, and their physical mixture

interactions between the drug and polymer in the physical mixture.

### DSC study

DSC study pure drug ciprofloxacin and in the physical mixture was studied. Figure 2 shows the thermal traces of ciprofloxacin polymer and their physical mixture. The ciprofloxacin comprised two endothermic peaks at 154°C and 318°C. The peak at 318°C related to the melting point of ciprofloxacin. The physical mixture of ciprofloxacin and polymers also showed two endothermic peaks at 139°C and 275°C. These peaks are slightly shifted to a lower side which suggested that there may be a weak interaction between ciprofloxacin and MXG. This could be due to possible electrostatic interactions between drug and polymer.

### Visual appearance

During preparation of *in situ* ocular gel of the ciprofloxacin drug, visual appearance of formulation on varying the concentration of polymer in drug has been observed. The visual appearance of ciprofloxacin ocular gel is displayed in Table 3. The visual appearance of various formulations was transparent. It depicted the uniformly distribution of drug in

formulation. All the formulations prepared were clear without any turbidity any suspended particles or impurities.

### Clarity

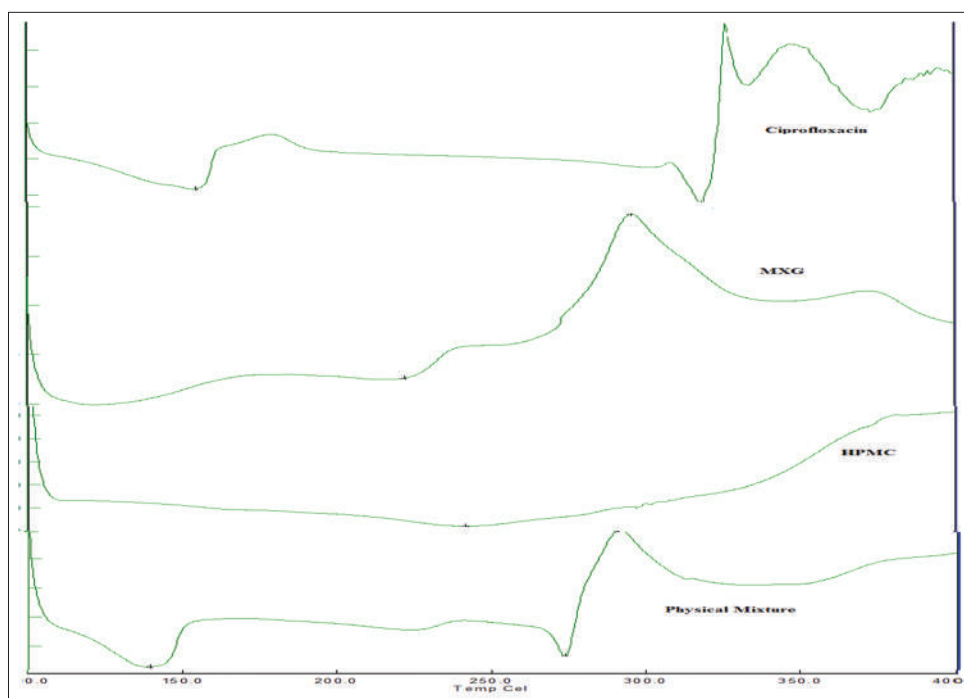
During preparation of *in situ* ocular gel of the ciprofloxacin drug, clarity of formulation on varying the concentration of polymer in drug has been observed. The clarity of ciprofloxacin ocular gel is displayed in Table 4. The clarity of various formulations was transparent. It depicted the uniformly distribution of drug in formulation.

### Measurement of pH

Factorial designed batches F1-F9 were prepared using various concentration of modified xanthan gum along with HPMC in different ratios. The pH of *in situ* gel solution was found to be around  $6.6 \pm 0.2$ – $6.9 \pm 0.08$  for all the formulations, as shown in Table 5. All formulation has pH which is an acceptable range for ophthalmic preparations.

### Gelation time

The gelation time measured for all prepared formulations is presented in Table 1. Gelation time of prepared *in situ* gel



**Figure 2:** Differential scanning calorimetry traces for ciprofloxacin, modified xanthan gum, HPMC, and their physical mixture

**Table 3:** Visual appearance analysis of formulations

Formulation code	Appearance
F1	Transparent
F2	Transparent
F3	Transparent
F4	Transparent
F5	Transparent
F6	Transparent
F7	Transparent
F8	Transparent
F9	Transparent

**Table 4:** Clarity analysis of formulations

Formulation code	Clarity
F1	Clear
F2	Clear
F3	Clear
F4	Clear
F5	Clear
F6	Clear
F7	Clear
F8	Clear
F9	Clear

solution was found to be around  $10.6 \pm 1.2$ – $32 \pm 0.8$  s. Table 5 shows for all the formulation it is cleared from the data shown that at constant modified xanthan gum concentration, gelation

time of different formulations decreased as the concentration of HPMC increased.

### Gelation temperature

Gel dosage forms are successfully used as drug delivery systems to control drug release and protect the medicaments from a hostile environment. The gelation involves the formation of double helical junction zones followed by aggregation of double helical segment to form three-dimensional (3D) networks by complexation with cations and hydrogen bonding with water. The temperature at phase transformation of the formulation solution to semisolid was noted which is called gelation temperature. Gelation temperature of gel for ophthalmic drug delivery should be around  $33.5^\circ\text{C}$  after dilution with Standard Tear Fluid. Ideal Gelation temperature of gel for ophthalmic drug delivery must be free flowing at low temperature, after contacting with ocular surface must be converted into semisolid and in the presence of maximum lachrymal secretion it must be in gel phase and to obtain a sol-gel transition temperature suitable for ocular application, i.e., near to corneal temperature  $35^\circ\text{C}$ . HPMC in aqueous solution has a low viscosity at low temperature and converted to gel after an increase in temperature. Under these circumstances, the gelation temperature will be lower than room temperature. Gelation temperature of prepared *in situ* gel batches was found to be around  $30.6 \pm 0.4$ – $35 \pm 0.8^\circ\text{C}$ , as shown in Table 5.

Formulation with maximum gelling temperature and minimum drug release. The maximum gelling temperature ensures enough time to administer the formulation while

**Table 5:** Evaluation of the various parameters of *in situ* gel formulations of ciprofloxacin

Formulation code	Modified xanthan gum %w/v	HPMC %w/v	pH	Gelation temp (°c)	Gelation time (s)	% Drug content
F1	1	0.5	6.7±0.2	34.3±0.4	32±0.8	98.12±0.04
F2	1	1	6.8±0.2	32±0.8	23.3±1.2	94.85±0.06
F3	1	1.5	6.8±0.1	35.3±0.4	21±0.8	96.35±0.047
F4	1.2	0.5	6.6±0.2	33±1.4	19±0.8	97.51±0.05
F5	1.2	1	6.6±0.09	35±0.8	15±0.8	97.99±0.04
F6	1.2	1.5	6.9±0.08	34±2.1	14±0.81	99.56±0.083
F7	1.5	0.5	6.8±0.08	30.6±0.4	30±0.816	94.9±0.08
F8	1.5	1	6.8±0.21	34.6±1.2	12.3±1.2	99.41±0.094
F9	1.5	1.5	6.5±0.08	32±0.8	10.6±1.2	93.09±0.057

**Table 6:** ANOVA response for the surface model of gelation temperature and viscosity

Response model	Sum of square	Degree of freedom	Mean square	F-value	P-value	R square	Ade. Precision	CV %
Gelation Temperature	37.23	12	7.02	5.66	Significant	0.8017	7.2	3.06
Viscosity	1.820E+05	12	3634	303	Significant	0.9954	59.7	1.36

ANOVA: Analysis of variance

minimum drug release ensures the release of drug for a longer period of time.

Statistical analysis data suggested quadratic model for response gelation temperature. The polynomial equations were found to be statistically significant ( $P < 0.01$ ), as determined using analysis of variance (ANOVA), as shown in Table 6.

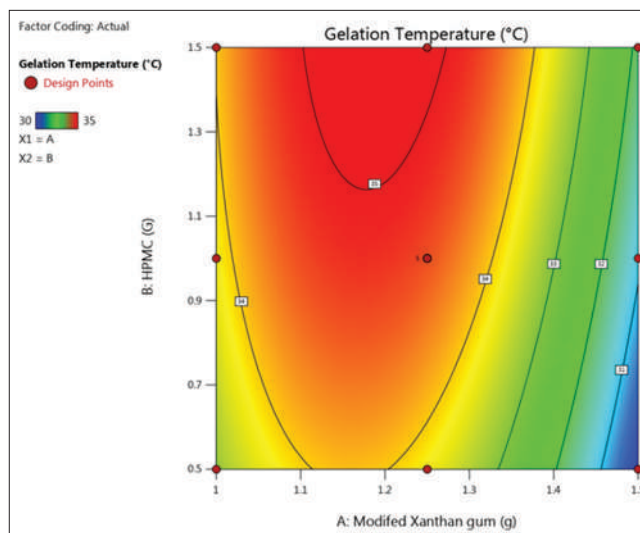
Impact of both the polymers on Gelation temperature was shown in the form of Polynomial equation below.

$$\text{Gelation temperature} = +34.62 - 1.33A + 0.6667B + 0.2500AB - 2.17A^2 - 0.1724B^2$$

3D response surface plots and contour plot showing the effect of formulation variables on gelation temperature are depicted in Figures 3 and 4. From the plots, as shown in Figures 3 and 4, it was concluded that there was a significant effect of modified xanthan gum and HPMC concentration on the gelation temperature. HPMC shows more pronounced effect than modified xanthan gum. Impact of both the polymers on Viscosity was shown in the form of Polynomial equation below.

### Drug content

The percentage of drug content for all formulations was determined and shown in Table 5. The drug content was found to be in the range of  $81.56 \pm 0.083 - 93.09 \pm 0.057\%$  for all the formulations indicating a uniform distribution of the drug.



**Figure 3:** Contour plot showing the influence of modified xanthan gum ... (X1) and... HPMC (X2) concentration on the gelation temperature

### Syringeability study

All the developed formulations were tested syringe ability through 21-gauge needle they passed through syringe.

### Viscosity (mpas)

The viscosity of the solutions was measured with respects to temperature at 10 rpm, 20 rpm, 50 rpm, and 100 rpm using appropriate spindle and results are shown in Table 7. It was found that increasing the concentration of the polymer

resulted in a significant increase in viscosity. Modified xanthan gum had a more pronounced effect on the viscosity than HPMC at the studied ranges.

As shown in Table 7, the viscosity was directly dependent on the polymeric content of the formulations viscosity which was studied at different RPM. All gel formulations exhibited a characteristics pseudoplastic (shear thinning) flow behavior at room temperature. The viscosity increased significantly with increasing concentration of modified xanthan gum and HPMC For all tested formulations. This may be explained by the fact that any incremental increase in the shear rate results in the alignment of the polymer chains polymer to each other along their axes in the direction of flow, thus reducing the internal resistance of the material and lowering viscosity.

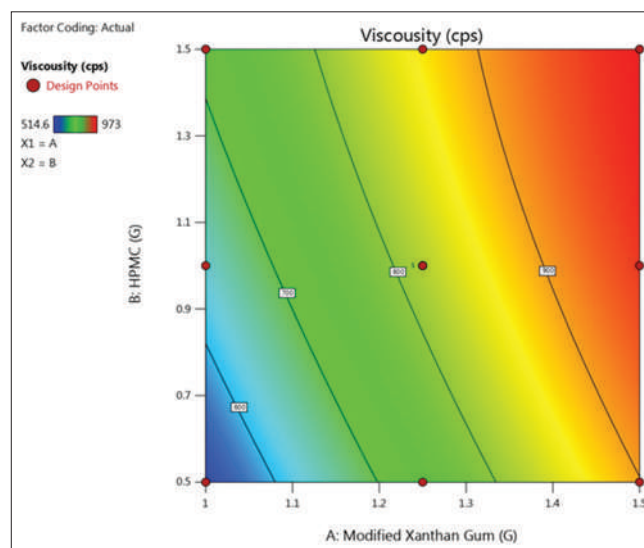
The viscosity of the formulations decreases with increasing shear rate in the presence of hydrophilic additives, for example, the viscosity of *in situ* gel formulations at 10 rpm.  $990 \pm 4.5$ , however, further increase in the shear force at 100 rpm resulted in significant decreases in the viscosity of the formulations  $973 \pm 1.6$  mPas, Table 7.

The higher concentration of the modified xanthan gum and HPMC among the developed formulations F9 gives good results which are the selected as optimized batch. 3D response surface plots and contour plot showing the effect of formulation variables on viscosity are depicted in Figures 5 and 6. From the plots, as shown in Figures 5 and 6, it was concluded that there was a significant effect modified xanthan gum and HPMC concentration on the on the viscosity. Modified xanthan gum shows more pronounced effect than HPMC. Mathematical relationship in the form of polynomial equation for the measured response % viscosity:

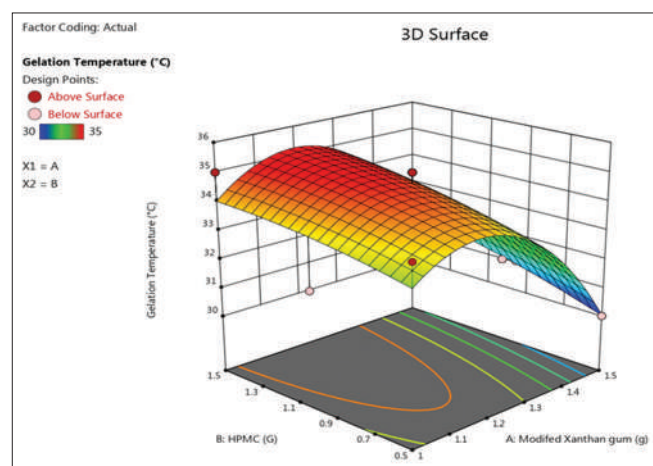
$$\text{Viscosity} = +821.66 + 156.67A + 65B - 30.50AB - 28.79A^2 - 16.79B^2$$

**Table 7: Rheological profile of *in situ* gelling systems**

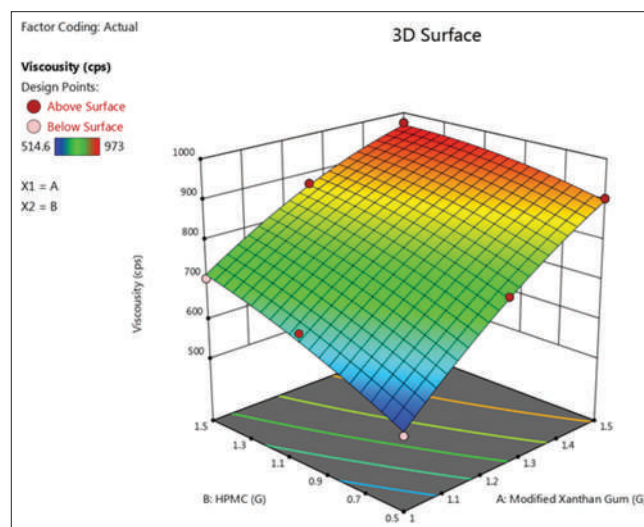
Batch	Viscosity (mpas)			
	10 RPM	20 RPM	50 RPM	100 RPM
F1	594±2.1	582.3±2.0	571±2.9	514.6±2.4
F2	695±2.4	684.4±2.8	679±7.4	656.6±3.0
F3	753±2.4	736±2.1	721±2.6	705±3.7
F4	772±2.1	762±2.1	753±2.4	744.6±2.8
F5	842.6±2.0	836.6±1.2	829.3±1.2	820±4.0
F6	895.3±3.0	893.6±2.6	889.6±1.2	874±2.4
F7	932.6±2.0	923.3±1.6	917.6±2.0	904±3.3
F8	957±1.6	951.3±1.2	942.3±2.0	938±2.4
F 9	990±4.5	983.6±1.2	979.6±1.2	973±1.6



**Figure 5:** Contour plot showing the influence of modified xanthan gum ... (X1) and... HPMC (X2) concentration on the viscosity



**Figure 4:** Three-dimensional response surface plot showing the influence of modified xanthan gum ... (X1) and... HPMC (X2) concentration on the gelation temperature



**Figure 6:** Three-dimensional response surface plot showing the influence of modified xanthan gum ... (X1) and... HPMC (X2) concentration on the on the viscosity



This study suggest feasibility of the model in the development of *in situ* Gel of Ciprofloxacin since predicted values agreed well with the experimental values,.

### In vitro drug release study

Figure 7 shows the release profile of different *in situ* gel formulations modified xanthan gum and HPMC retarded the dissolution of the drug in a concentration-dependent manner as the release rate of ciprofloxacin decreased with increasing modified xanthan gum and HPMC ratio. Batches containing maximum gelling temperature and minimum drug release. The maximum gelling temperature ensures enough time to administer the formulation while minimum drug release ensures the release of drug for a longer period of time.

Drug release in case of *in situ* gel of ciprofloxacin was found to be 91.59% drug release in 12 h. Thus, the *in vitro* dissolution test indicated the sustained release nature of *in situ* gel of ciprofloxacin. *In vitro* release study indicated that the release of drug varied according to the type and concentration of polymers. The results further showed that the amount of the drug released in the 1<sup>st</sup> h decreased with the increase in both the polymer concentration and this pattern continued till the entire duration of the study, as shown in Figure 7. *In vitro* drug release studies showed a polymer concentration-dependent decrease in drug release. As the polymer concentration decreases, the release rate profile increases.

### Results of ANOVA study

ANOVA was applicable to determine the effect of variables and their interaction. The regression model was used to develop contour plots of independent factors. Responses observed for nine formulations were fitted to Design Expert software latest version 12 outcome of ANOVA as shown in Table 6.

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

### Mathematical modeling and release kinetics

To interpret the mechanism and kinetics of drug release, the result outcome of *in vitro* drug release study was applied with different kinetic equations. The *in vitro* release data were subjected to goodness of fit test by linear regression analysis according to zero-order, first-order kinetic equations, Higuchi equation, Korsmeyer-Peppas, and Hixson-Crowell models to ascertain the release. As per Table 8, the results were suggested that the value of of  $R^2$  (Regression coefficient) was obtained linear in case of Higuchi Kinetics as compared to zero-order

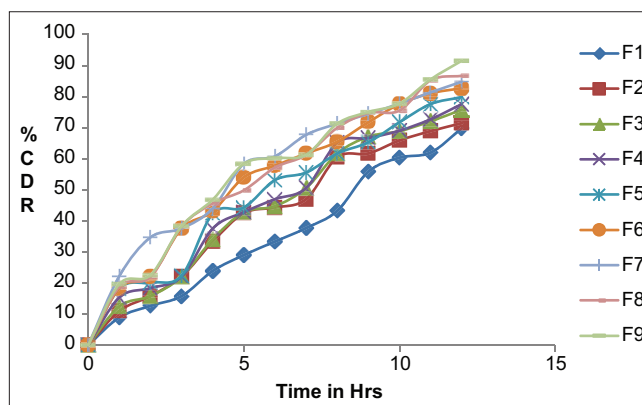


Figure 7: *In vitro* drug release data of optimized batches of ciprofloxacin

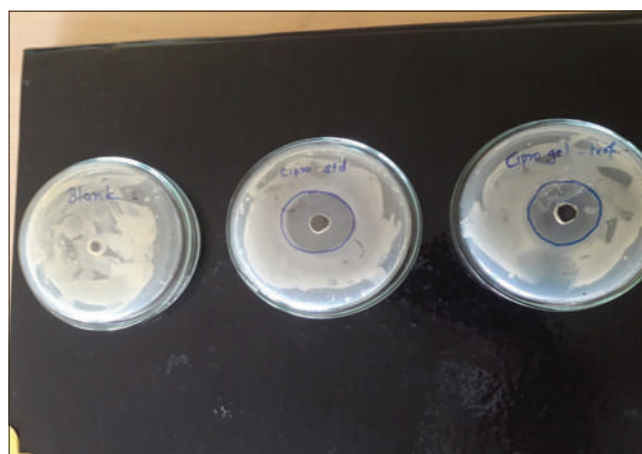


Figure 8: Zone of inhibition of *in situ* gel and ciprofloxacin against of *Pseudomonas aeruginosa*

Table 8: Release mechanism of optimized formulation

Batch code	Zero-order	First-order	Higuchi Model	Korsmeyer-Peppas	<i>n</i>
	$R^2$	$R^2$	$R^2$	$R^2$	
F8	0.9788	0.8826	0.9958	0.9766	1.96

and first-order kinetics for all formulations. In the present study, the coefficient of determination ( $R^2 = 0.9958-0.8826$ ) was found to be much closer to one and mechanism for drug release is diffusion.

### Antimicrobial activity

Microbial keratitis is a serious complication of eye. Many different microbial strains have been isolated from microbial keratitis, in which near about two thirds are Gram-negative bacteria. In this, *P. aeruginosa* are most notable strain. The antibacterial study of ciprofloxacin gel is shown in Figure 8. The zone of inhibition of *in situ* gel and ciprofloxacin was found to be 25 and 28 mm against *P. aeruginosa*. Distilled water is used as blank and has no zone of inhibition.

## Selection of optimized formulation

To optimize a formulation with better properties, Design Expert\_12 was used to target a formulation with maximum gelling temperature and minimum drug release. Formulation F8 selected optimized batch since maximum gelling temperature  $34.6 \pm 1.2^\circ\text{C}$  and minimum drug release 91.59% drug release ensures the release of drug for a longer period of time.

## CONCLUSION

Ciprofloxacin Hydrochloride is fourth generation fluoroquinolone broad-spectrum antibiotic has physicochemical properties required to permeate cornea. *In situ* gel of ciprofloxacin hydrochloride for ophthalmic drug delivery was prepared using modified Xanthan gum and HPMC and it was characterized for various parameters. FT-IR and DSC study suggest no interaction between Ciprofloxacin and polymers. The clarity of all formulations was transparent. Prepared batches of *in situ* gel show acceptable range pH  $6.6 \pm 0.2$ – $6.9 \pm 0.08$  for ocular use. Gelation temperature. *in situ* gel batches were found to be  $30.6 \pm 0.4$ – $35 \pm 0.8^\circ\text{C}$ . Gelation time of prepared *in situ* gel solution was found to be around  $10.6 \pm 1.2$ – $32 \pm 0.8$  S. It was found that increasing the concentration of the polymer resulted in a significant increase in viscosity. Modified xanthan gum had a more pronounced effect on the viscosity than HPMC at the studied ranges. Formulation F8 selected optimized batch has maximum gelling temperature  $34.6 \pm 1.2^\circ\text{C}$  and minimum drug release 91.59% drug release. Optimized formulation gave satisfactory results in terms of antimicrobial activity. The optimization study was successfully conducted using 32 factorial designs. Developed *in situ* gelling systems will be viable alternative to conventional eye drops and ointment in terms of ease of administration with added benefits of sustained drug released which may ultimately results into improved, bioavailability & patient compliance, So we finally concluded that formulated Ciprofloxacin Hydrochloride *in situ* gelling system shows a promising approach toward the treatment of bacterial infections.

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