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FORMULATION AND EVALUATION OF pH TRIGGERED *IN SITU* GELLING OPHTHALMIC SUSTAINED DRUG DELIVERY SYSTEM OF CIPROFLOXACIN HYDROCHLORIDE

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The lower residence contact time of drug, higher tear turnover, limited surface area of contact and impermeability of corneal epithelium layer are the reasons of poor bioavailability of conventional ophthalmic dosage forms. The purpose of this study is to formulate ciprofloxacin hydrochloride (HCl) in situ gelling ophthalmic drug delivery system based on pH of tear fluid for better bioavailability and minimising frequent instillation. This system includes pH sensitive polymer (Carbopol 934p) as a gelling agent and HPMCK15M as a viscosity enhancer which are instilled as a drop and undergo sol-gel transition in the cul-de-sac. Central composite design with two independent factors (Carbopol 934p and HPMCK15M) followed by contour plot and surface response was used to optimise formulation (Optimized). The drug was complexed with Indion® 254 to avoid drug-Carbopol 934p instantaneous incompatibility. The rheological study of Optimized exhibited pseudoplastic behaviour with adequate viscosity at higher pH (7.40 or above). It provided prolonged drug release time (8 hours). It followed Krosmeyer–Peppas model with non-Fickian diffusion. Theocular irritancy based on hen's egg test-chorioallantoic membrane (HET-CAM) technique suggested that it had acceptable mild irritancy (0.33, 0.66 and 0.66 out of 3 at 6th, 7th and 8th hours, respectively). It retained its antimicrobial response towards Pseudomonas aeruginosa (ATCC 10145) and Staphylococcus aureus (ATCC 25903). The results of gelling capacity, rheological behaviour and in vitro release studies of $F_{optimized}$ demonstrated that Carbopol 934p–HPMCK15M composite can help to enhance ocular bioavailability.

Keywords: *In situ* gelling, pH triggered, Ciprofloxacin HCl, Carbopol 934p, HPMCK15M

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INTRODUCTION

The common method of ocular drug delivery is topical administration of ophthalmic drops into the lower *cul-de-sac*. Such drops are outflow quickly due to the eye blinking reflux and the pre-corneal region returns to maintain resident volume of around 7 µl. The available concentration of drug in pre-corneal fluid is the driving force for passive transport of drug across the cornea.

The cornea is an optically transparent tissue and is a protective barrier (multiple layers) to the interior of the eye though it has a small surface area (~1 cm^2). Therefore, it is a major barrier for traditional topical drug delivery in the treatment of anterior segment diseases such as glaucoma, keratitis and bacterial and viral infections. Pre-corneal drainage, tears washout and limited contact time are major challenges to the anterior segment drug delivery upon topical administration. To be clinically effective, topical formulation has to possess a balance between the hydrophilicity and lipophilicity with higher contact time (Kansara, Hao and Mitra 2007). Design of modern ocular drug delivery systems is based on the drug application pathways, absorption mechanism and the overall ocular pharmacokinetic/ pharmacodynamic profile. Various ocular drug delivery such as hydrogels, micro-particles, nanoparticles, liposomes, insert, *in situ* gelling system and other colloidal systems, as well as solid inserts and shields or surgically applied polymeric implants have been proposed for prolonging the release of drugs and enhancing corneal bioavailability.

The progress has been made in gel technology for the development of droppable gel. They are liquid upon instillation and undergo phase transition in the ocular *cul-de-sac* to form visco-elastic gel that provides a response to environmental changes. Ideally, an *in situ* gelling system should be a low viscous, free flowing liquid to allow for reproducible administration to the eye as drops, gel formed following phase transition should be strong enough to withstand the shear forces in the *cul-de-sac* and demonstrates long residence time. Three methods, viz. change in pH, change in temperature and ion activation, have been used for phase transition in the eye surface.

In human body, there are remarkable changes in pH that can be used to direct therapeutic agents to a specific body area, tissue or cell compartment. Owing to wide variations in the pH value of the physiological fluids, sol–gel transitions induced by pH changes seem to be an ideal approach for enhancing the pharmacological efficacies of the topical drug delivery, especially ophthalmic and intravaginal applications. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. Swelling of hydrogel increases as the external pH increases in case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pH-sensitive polymers are based on polyacrylic acid (PAA) (Carbopol® or carbomer) or its derivatives. Likewise, polyvinylacetal diethylaminoacetate (AEA) solutions with a low viscosity at pH 4 form hydrogel at neutral pH condition. Other polymers like cellulose acetate phthalate latexes (CAP), polymethacrylic acid (PMA), polyethylene glycol (PEG) and pseudo latexes also have been used as pH sensitive system to achieve gelation (Almeida *et al*. 2013). In this study, authors aim to develop and evaluate pH triggered *in situ* gelling ophthalmic drug delivery approach to prolong the effect of ciprofloxacin hydrochloride (HCl).

MATERIALS AND METHODS

Materials

Ciprofloxacin HCl, working reference standard, excipients (Carbopol 934p, HPMCK15M, benzalkonium chloride (BKC), disodium EDTA, resins (Indion®254, Indion®214) and chemicals used in this study were obtained from Nepal Pharmaceuticals Laboratory (NPL) Pvt. Ltd., Jeetpur, Birgunj as gift samples. Marketed product (Microflox) was purchased from local retail pharmacy and was used as a reference product for data analysis.

Analytical Method Development

A robust high performance liquid chromatographic (HPLC) based analytical method has been developed and validated for the quantification of ciprofloxacin HCl in pH triggered *in situ* gelling formulation based on validation parameters viz. linearity, specificity, accuracy, repeatability, recovery, robustness, limit of quantification and limit of detection as per Q2B validation of analytical procedures (ICH 1996).

Chromatographic Method

The chromatographic analysis of the drug was carried out in HPLC (Shimadzu Prominence® ultrafast liquid chromatography [UFLC] Nexera liquid chromatograph) with the conditions as in Table 1.

Table 1: Chromatographic system.

	System	Condition
1.	Mobile phase	Acetonitrile: Millipore water: Triethyl amine = 25:75:0.1 with pH adjustment 4.0 ± 0.05 by using orthophosphoric acid
2.	Flow rate	1 ml/minute
3.	Column	Purospher® STAR RP-8 end capped (5 µm) octylsilane chemically bonded to porous silica (C8) [150 mm x 2.5 ml x 4.6 mm id], manufactured and certified on 27 May 2013 by Merck kGaA, Darmstadt, Germany
4.	Detector	278 nm
5.	Injection volume	25 µl
6.	Temperature	35°C

Buffer System Selection

In order to ensure product stability and desired drug solubility for ciprofloxacin HCl in *in situ* gelling system, various buffer systems viz. acetate buffer IP (pH 4.0, 4.5, 5.0, 5.5, 6.0), phosphate buffer USP (pH 4.0, 5.0, 6.0, 6.5), citrophosphate buffer BP (pH 4.0, 5.0, 6.0, 6.5) were used. The solubility of drug in these buffer systems was carried out at a concentration of 0.3% w/v.

Drug-Excipients Compatibility Studies

Compatibility studies were carried out by isothermal stress testing (IST) technique. In this technique, drug and different excipients were weighed in unimolar ratio and then was transferred accurately into glass vials (5 ml; *n* = 2). The composite systems were mixed on a vortex mixer for 2 minutes. Then water (10%) was added to each of the vials and the drug-excipient blend was further mixed with a both end heat-sealed glass capillary. Each vial was sealed using a teflon-lined screw cap and stored at 50 ± 0.5°C in hot air oven. Similarly, control sample were prepared and stored under refrigerated condition for comparative study. The samples were periodically examined for any unusual colour changes. After three weeks of storage under above condition, samples were quantitatively analysed using validated HPLC method (Table 1) with reference to refrigerated control sample and qualitatively by Fourier transform infrared spectroscopy (FTIR) (Dong and Choi 2008; Nihar, Lila and Sujata 2011; Kannan 2013). The qualitative data from FTIR and quantitative data from HPLC were compared for drug-excipient compatibility study.

FTIR Study of Ciprofloxacin HCl

Ciprofloxacin HCl (10 mg) was diluted with potassium bromide to get around 1000 mg of mixture and triturated to ensure sample homogeneity. For each sample, FTIR spectra and purity index were recorded in the wave number ranged between 2000 cm^{-1} and 400 cm^{-1} averaging 45 scans per sample using a resolution of 3.86 cm^{-1} (FTIR-21 prestige spectrophotometer, Shimadzu). The IR solution software was used for data collection and qualitative study.

Preparation of Resinate (Drug-resin Complex)

In this technique, the activated resins (Indion® 214 and 254) were dried and then grounded to fine powder; typically in the range of 40 µm–150 µm. Resins were mixed with a drug solution by batch process by allowing sufficient time for drug loading and equilibrium of ion exchange process. The resin/fluid slurry was filtered, filtrate washed and then dried in an oven at 60 ± 0.5°C (Jain *et al*. 2008).

Drug Loading Efficiency Study in Resinates

Ion exchange activated resins (Indion® 214 and 254) were separately dispersed uniformly in water using an overhead stirrer for 15 minutes in resin: drug equals to 1:1 and 2:1. The ciprofloxacin HCl was then added to the dispersion while stirring and this continued until equilibrium of resin and complex were achieved. Periodically sample were withdrawn and centrifuged at 2000 rpm (REMI-R-8CBL centrifuge). The spectrum of supernatant was scanned in UV-1800 and λmax was observed at 274 nm which was used for quantification of uncomplexed ciprofloxacin HCl using Equation (1).

$$\text{The bound drug inresinate system} = \frac{\text{Total amount of drug in drug slurry system} - \text{Amount of drug in supernatant}}{\text{Actual amount of drug added}} \times 100 \quad (1)$$

FTIR Study of Resinates

FTIR spectra of resinate complex of ciprofloxacin HCl and resin **(**Indion®214 and 254) were recorded in the wave number ranged between 2000 cm^{-1} and 400 cm^{-1}, averaging 45 scans per sample using a resolution of 3.86 $cm^{-1.}$

Preparation Method of *In situ* Gelling System

Buffer salt, sodium acetate trihydrate, disodium edetate and BKC were dissolved in water (75 ml). Then, HPMCK15M was added to the above solution and allowed to hydrate for 30 minutes. Carbopol 934p was dispersed in the solution and allowed to hydrate overnight with stirring. Resinate equivalent to 0.3% w/v of ciprofloxacin was added to above solution under stirring in order to make uniform suspension of resinate. The pH of the system was adjusted to 4.5 ± 0.2 using glacial acetic acid/0.5 N NaOH. After pH adjustment, prepared formulation were dispensed in amber glass vials (5 ml), closed with grey butyl rubber closures and sealed with aluminum caps. Finally formulations were terminally sterilised in autoclave (Thermolab) at 121.5°C (15 psi) for 20 minutes (Jain *et al*. 2008).

***In situ* Gelling System Characterisation**

pH of the formulation

The pH of the formulation was measured by calibrated pH meter (Hanna).

In situ gelling capacity

The gelling capacity of formulation was assessed by placing a drop of the system in a vial containing 1 ml of artificial tear fluid (ATF), freshly prepared with maintaining temperature at 37 ± 0.5°C under Neo lab heating block system. The composition of the ATF contains (NaCl = 0.670 g, $NaHCO_3$ = 0.20 g, $CaCl_2.2H_20$ = 0.008 g) with purified water quantum satis (qs) 100 ml (Gupta *et al*. 2007; Jain *et al*. 2008).

Assay

The assay of ciprofloxacin HCL in *in situ* gelling system was carried by validated HPLC method (Table 1).

Standard preparation

Ciprofloxacin HCl RS (equivalent to 60 mg) was taken into volumetric flask (100 ml) and distilled water (60 ml) was added. The solution was sonicated for 10 minutes followed by shaking for 15 minutes in mega stirrer at 500 rpm. The volume was made up to 100 ml with distilled water. The reference standard solution was diluted further with 0.1 M HCL in order to make final concentration of 60 µg/ml.

Sample preparation

In order to estimate ciprofloxacin content in formulation, 2 ml of *in situ* gel formulation was pipetted accurately and then transferred into volumetric flask (100 ml). The resinate in the formulation was dissolved in 0.1 M HCl in ultrasonic bath for 30 minutes followed by shaking for 30 minutes in mega stirrer at 500 rpm. The volume was made up to 100 ml with 0.1 M HCl. The solution was filtered through Whatman No.1 discarding first few filtrates.

Procedure

Each of standard reference and sample solutions (25 µl) were injected in UFLC-Nexera with chromatographic condition (Table 1). The corresponding responses in terms of area were noted to deduce the result in comparative manner (Equation 2).

$$\text{Quantity of drug per ml} = \frac{\text{sample area}}{\text{std area}} \times \frac{\text{std weight}}{\text{std dilution factor}} \times \frac{\text{sample dilution factor}}{\text{sample volume}} \times \frac{331.35}{367.82} \times \frac{\text{std potency}}{100} \times \frac{100 - \text{water}}{100} \times 100 \quad (2)$$

In vitro release study

In vitro evaluations of formulation were carried out with modification of USP type-I dissolution apparatus. The lower portion of basket was wrapped with Spectra/por® 7 (cellophane membrane). The *in vitro* release study was carried out using ATF as a medium (100 ml) at 50 rpm for 8 hours. Spectra/Por® 7 (cellophane membranes) was activated by soaking in distilled water for 30 minutes and then overnight in ATF before wrapping at the base of basket (Bottari *et al.*1974; Desai and Blanchard 1998).

Standard preparation

Ciprofloxacin HCl RS equivalent to 30 mg of ciprofloxacin was taken in a volumetric flask (100 ml) and ATF (60 ml) was added. The solution was sonicated for 10 minutes followed by shaking for 15 minutes in mega stirrer at 500 rpm. The volume was made up to 100 ml with same medium. The reference standard solution was diluted further with medium in order to make final drug concentration of 30 µg/ml.

Sample preparation

The formulated sample (1 ml) was placed in basket wrapped with cellophane membrane on basement surface. In vitro study was assessed by withdrawing sample (1 ml) at an interval of 1 hour for 8 hours with replenishment by ATF under thermostatically controlled hydrodynamics condition.

Procedure

Ciprofloxacin RS and sample solutions, each 25 µl, were injected separately in UFLC-Nexera with chromatographic condition mentioned in Table 1. The corresponding responses in terms of area were noted to deduce the result of percentage drug release in comparative manner with ciprofloxacin HClRS using Equation 3.

$$\%\ \text{drug release} = \frac{\text{sample area}}{\text{std area}} \times \frac{\text{std weight}}{\text{std dilution factor}} \times \frac{\text{sample dilution factor}}{\text{sample volume}} \times \frac{331.35}{367.82} \times \frac{\text{std potency}}{100} \times \frac{100 - \text{water}}{100} \times 100 \quad (3)$$

Rheological Study

The rheological study of the formulation were determined using the spindle (S64) at 25 ± 1°C for both sol (pH = 4.5 ± 0.2) and gel phase with increase in pH of the system. The developed formulations (pH = 4.5 ± 0.2) were poured into the beaker, the angular velocity was increased gradually from 1 rpm to 100 rpm at different angular velocities (1, 2, 2.5, 4, 5, 10, 20, 50,100 rpm). The angular velocity was reversed and average viscosity was calculated. The same formulation were again evaluated for viscosity determination in a similar fashion with increase in pH of the system (pH = 7.4 or above) by addition of 0.5 M NaOH using a Brookfield programmable DV-II+ Viscometer. The viscosity measured at both the conditions versus angular velocity was plotted for selected formulations to study rheological behaviour (Srividya, Cardova and Amin 2001).

Ocular Irritation Test

Ocular irritation study was carried out by modified hen‘s egg test or Huhner-embroynen-test-chorioallantoic membrane (HET-CAM) test. Fertilised hen’s eggs weighing between 50 g and 60 g were obtained from local poultry farm. They were candled to discard the defective ones. The selected eggs were incubated in a humidified incubator at a temperature of 37 ± 0.5°C for three days. The trays containing eggs were rotated manually in a gentle manner after every 12 hours. On day 3, egg albumin (3 ml) was removed by using sterile techniques from the pointed end of the egg. The hole was sealed by 70% alcohol sterilised parafilm with the help of sterilised spatula. The eggs were kept in the equatorial position for the development of chorioallantoic membrane (CAM) away from the shell. The eggs were candled on the 5th day of incubation, and every day thereafter non-viable embryos were removed. On 10th day, a window (2 cm × 2 cm) was made on the equator of the eggs through which formulations (0.5 ml) were instilled. A 0.9% NaCl solution and dioctyl sodium sulphosuccinate were used as negative and positive controls, respectively (Velpandian *et al*. 2006).

Antimicrobial Efficacy Testing

The antimicrobial efficacy test of formulated *in situ* gelling system was carried out using materials mentioned in Table 2.

Table 2: The material for antimicrobial efficacy testing.

	Materials
1.	Muller Hinton agar (MHA) media
2.	Sterile cotton swab
3.	Petri plates
4.	Strain of *Staphylococcus aureus* (ATCC 25903)
5.	Strain of *Pseudomonas aeruginosa* (ATCC 10145)
6.	Antibiotic discs
7.	70% isopropyl alcohol (IPA)
8.	Macfarland standard
9.	0.9% NaCl (normal saline)

Preparation of Muller Hinton Agar Media (MHA) and MHA Petri Plates

MHA (38 g) was suspended in 1000 ml distilled water and then heated gently to dissolve the medium completely. The dissolve media was then autoclaved at 15 lbs (121°C) for 15 minutes. After autoclaving, 25 ml of MHA media was poured in sterilised eight petri plates under horizontal laminar flow cabinet with avoiding air entrapment. The MHA in petri plates were solidified for the purpose of inoculums culture on its surface (Mueller and Hinton 1941).

Inoculums Preparation

In order to prepare inoculums, strain of *Staphylococcus aureus* (ATCC 25903) and *Pseudomonas aeruginosa* (ATCC 10145) were sub-cultured in soybean-casein digest agar media (SCDA). SCDA (40 g) in distilled water (1000 ml) and then heated gently to dissolve the medium completely. The dissolve media was then sterilised by moist heat sterilisation (autoclave) at 15 lbs (121°C) for 15 minutes. The strain microorganisms were sub-cultured in SCDA slant media with the help of sterilised wire loop. Finally, slants were incubated at 36 ± 1°C for 2–3 days. After 2–3 days, the media was found turbid due to growth of microorganisms (Bauer *et al*. 1966).

Inoculate Transfer to MHA Petri Plates

After adequate microbial growth of sub-cultured strain of *Staphylococcus aureus* (ATCC 25903) and *Pseudomonas aeruginosa* (ATCC 10145) in SCDA, each was homogenously mixed in vortex mixer for 2–3 minutes. The turbidity of liquid suspension was compared with 0.5 McFarland standards (Murray, Baron and American Society for Microbiology 2003) under adequate light source. The solution turbidity was diluted with sterile 0.85% w/v saline solution and turbidity was made comparable to standard one, which depicted the bacterial count within a desirable limit. The MHA plates were inoculated with strain of *Staphylococcus aureus* (ATCC 25903) and *Pseudomonas aeruginosa* (ATCC 10145) on its surface with sterile cotton swab in aseptic condition. Antibiotics discs were prepared by diffusing ciprofloxacin HCl solution over (0.45 µm) aseptic membrane filter paper with the help of micropipette. The discs were allowed for complete soak. During efficacy testing disc having different concentrations of reference standard (3 µg, 30 µg, 90 µg and 300 µg) and test samples were prepared. After discs preparation, each concentration discs were

dispensed over MHA plates separately for both strains of bacteria aseptically. The MHA plates were incubated at 36 ± 1°C for two days for the antibiotics inhibitory effect study. The antimicrobial efficacy testing of optimised batch was compared with marketed ciprofloxacin eye drop *(Microflox)* on the basis of diameter of zone of inhibition (Bauer *et al*. 1966; Murray and Baron 2007).

Formulation Optimisation

Contour plot and response surface methodology (RSM) were used to optimise the formulations based on polynomial equation (Equation 4) that show interaction and quadratic terms for release of drug from gelling system generated for all the response variables using multiple linear regression analysis (MLRA) approach.

$$Y = B_0 + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{22}X_1^2 + B_{12}X_1X_2 \quad (4)$$

where,
Y = dependent variable
B_0 = intercept representing the arithmetic average of thirteen batches
B_1 = estimated coefficient for factor X_1
X_1 and X_2 = coded levels of the independent variables

Mathematical Modeling for Drug Release Kinetics

The following models have been tested for release data of the formulations:

Zero order kinetics

Zero order kinetics is the one whose rate is independent of the concentration of drug undergoing reaction (Equation 5) (Murray and Baron 2007).

$$C = k_0t \quad (5)$$

where,
C = concentration of drug at time = t
k_0 = zero order release constant

First order kinetics

First order kinetics is the one whose rate is directly proportional to the concentration of drug undergoing reaction. It is also called linear order kinetics because of proportionality between rate of reaction and the concentration of drug (Equation 6),

$$\log C = \log C_0 - \frac{K_1t}{2.303} \quad (6)$$

where,
C = concentration of drug at time (t)
C_0 = concentration at t = 0

Higuchi equation

Higuchi square root model is based on Fick's laws of diffusion and is applicable to porous hydrophobic drug delivery system in homogenous and granular matrices. The model is expressed as Equation (7),

$$f_t = Q = \sqrt{\frac{D_\varepsilon}{\tau}(2C - \varepsilon C_s)C_s t} \quad (7)$$

where
Q = amount of drug released in time *t* by surface unity
C = initial concentration of the drug
ε = matrix porosity
τ = tortuosity factor of the capillary system
C_s = drug solubility in the matrix
D = diffusion constant of the drug molecules in that liquid

Korsmeyer-Peppas model (power law)

The semi-empirical equation developed by Korsmeyer and Peppas in 1983 is based on the Fick's laws of diffusion. It can be used to analyse data of controlled release water soluble drugs from polymers. According to this model drug release kinetics, the fractional release of drug is exponentially related to release time, and is expressed as Equations (8) and (9) (Siepmanna and Peppas 2001):

$$f_t = at^n \quad (8)$$

where,
a = constant incorporating structural and geometric characteristics of the dosage form
n = release exponent, indicative of the drug release mechanism
t = time
f = function is fractional release of drug ($\frac{M_t}{M_\infty}$)

Under some experimental situations release mechanism deviated from the Fick equation and follows an anomalous behaviour (non-Fickian). In these cases more generic equation can be used as:

$$\frac{M_t}{M_\infty} = at^n \quad (9)$$

Similarity and Dissimilarity Factor

Similarity factor

Similarity between the two products is assessed by using similarity factor (F_s). F_s is a logarithmic transformation of the sum-squared error of differences between the test T_j and reference products R_j over all points (Equation 10).

$$F_s = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{j=1}^{n} (Rj - Tj)^2 \right]^{-0.5} \times 100 \right\} \quad (10)$$

Where *n* is the sampling number, R_j and T_j are the % dissolved of reference and the test products at each time points *j*, respectively. f_s value higher than 50 and close to 100 show the similarity of the dissolution profiles.

Dissimilarity factor

The difference factor (F_d) measures the percent error between two curves over all time points (Equation 11).

$$F_d = \left[\frac{\sum_{i=1}^{n} (Rj - Tj)}{\sum_{i=1}^{n} Rj} \right] \times 100 \quad (11)$$

The percentage error is zero when the test and drug reference profiles are identical and increase proportionally with the dissimilarity between the two dissolution profiles. The F_d values should be close to 0 to be similar.

RESULTS AND DISCUSSION

Analytical Method Validation

Linearity

The area of responses versus concentrations (20, 40, 60, 80, 100 µg/ml) was plotted (Figure not shown). The plotted linear equation was Y = 79562.4X-255511 with correlation coefficient (R^2) of 0.9961.

Specificity

The developed analytical procedure had completely discriminates from its placebo with prominent peak at 278 nm having signal to noise (S/N) ratio of 100:1. The respective noise and drift upon 25 µl placebo injection were 141.85 and −1207.96, respectively. Similarly, the respective noise and drift upon 25 µl analyte injection were 382.36 and −165638.99, respectively.

Accuracy

The analytical method was found to be accurate across the different concentration level within linearity range. Similarly, the recovery of analyte at below and above 20% from assay level was within limit. The relative standard and standard error were below 2% (table not shown).

Precision

The procedure was found to be precise at assay level upon repetitive performance at different time interval within a day (intermediate precision) and day after (interday precision). The relative standard deviations at different conditions were below 2% indicated that process had precision (table not shown).

Detection limit

Based on standard deviation of responses and slope of calibration curve (figure not shown) the detection limit was found to be 0.1427 µg/ml.

Quantification limit

Based on standard deviation of responses and slope of calibration curve (figure not shown), the quantification limit was found to be 0.4759 µg/ml.

Robustness

As the % RSD of robustness below 2%, the robustness result upon deliberate variation of pH in mobile phase (± 0.05 unit), oven temperature (± 5°C), and organic solvent (± 5%), different columns (different lots and/or suppliers) were found within the limit (tables not shown) and hence the method had robustness.

Buffer system selection

Out of three buffer systems of different pH, acetate buffer IP (pH 4.5) (solubility = 50.59 mg/ml) was selected for formulation.

FTIR compatibility study

The drug-excipient composite stored under respective condition as mention in Table 3 were quantified by reverse phase (RP)-HPLC method. The drug content under both control and stressed conditions was found within a range of 97.09% to 101.42%. The quantity of drug remaining under stressed condition of 10% moisture at 50°C with reference to control sample suggested that there were no significant drug degradation phenomenon.

The qualitative studies of drug-excipient composite system were carried out by comparative study of FTIR absorbance bands with their purity index. The FTIR spectrum of ciprofloxacin HCl standard with addition of 10% moisture under IST exhibited additional absorbance band (1492.9 cm^{-1}) than spectrum of ciprofloxacin HCl reference standard [Figures 1(a) and 1(b)].

Table 3: Quantitative estimation of compatibility study by RP-HPLC

	Drug	Excipient	Ratio (drug-excipient)	Temperature	Moisture	Drug remaining (%)	
						Control[1]	Stressed[2]
1.	Ciprofloxacin	-	1:1	Refrigerated	-	99.78	
2.	Ciprofloxacin	-	1:1	50°C	+		97.87
3.	Ciprofloxacin	EDTA	1:1	Refrigerated	-	100.14	
4.	Ciprofloxacin	EDTA	1:1	50°C	+		98.70
5.	Ciprofloxacin	HPMCK15M	1:1	Refrigerated	-	99.55	
6.	Ciprofloxacin	HPMCK15M	1:1	50°C	+		97.09
7.	Ciprofloxacin	Carbopol 934p	1:1	Refrigerated	-	101.42	
8.	Ciprofloxacin	Carbopol 934p	1:1	50°C	+		99.99

Notes: [1] Drug-excipient blends without added water and stored in refrigerator (2°C–8°C);
[2] Drug-excipient blends with 10% added water and stored at 50°C for 3 weeks.

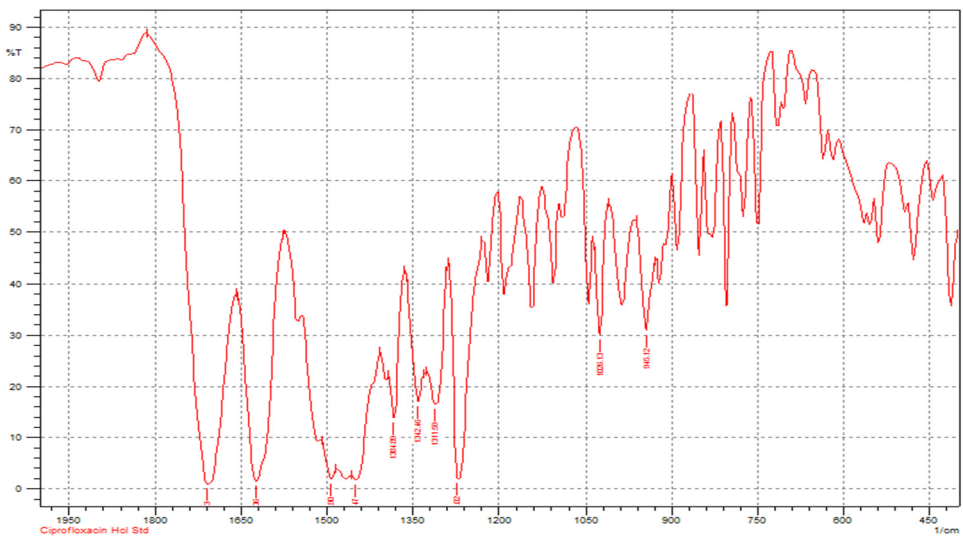

Figure 1(a): FTIR spectrum of ciprofloxacin HCl reference standard.

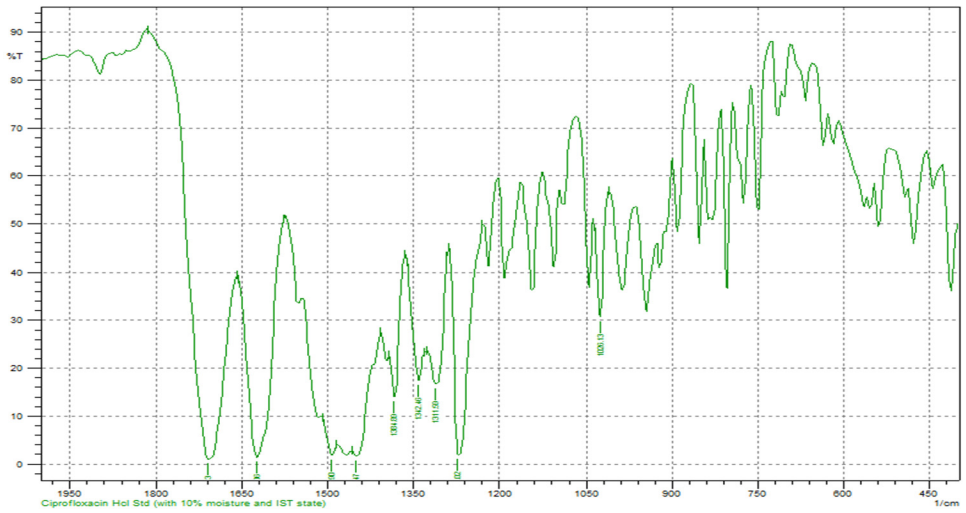

Figure 1(b): FTIR spectrum of ciprofloxacin HCl with 10% moisture under IST condition.

The FTIR spectrum of refrigerated ciprofloxacin-HPMCK15M exhibited absorbance bands in 412.77, 478.35, 540.07, 802.39, 921.97, 945.12, 987.55, 1026.13, 1045.42, 1087.85, 1141.86, 1180.44, 1290.01, 1273.02, 1311.59, 1342.46, 1384.89, 1450.47, 1492.9, 1624.06 and 1708.93 cm^{-1}. Similarly, unimolar mixture of ciprofloxacin-HPMCK15M with addition of 10% moisture under IST exhibited absorbance bands in 412.77, 443.63, 478.35, 540.07, 748.38, 802.39, 829.39, 852.54, 891.11, 921.97, 945.12, 987.55, 1026.13, 1045.42, 1091.71, 1141.86, 1192.01, 1219.01, 1269.16, 1311.59, 1342.46, 1384.89, 1450.47, 1492.9, 1624.06 and 1708.93 cm^{-1} [Figures 1(c) and 1(d)].

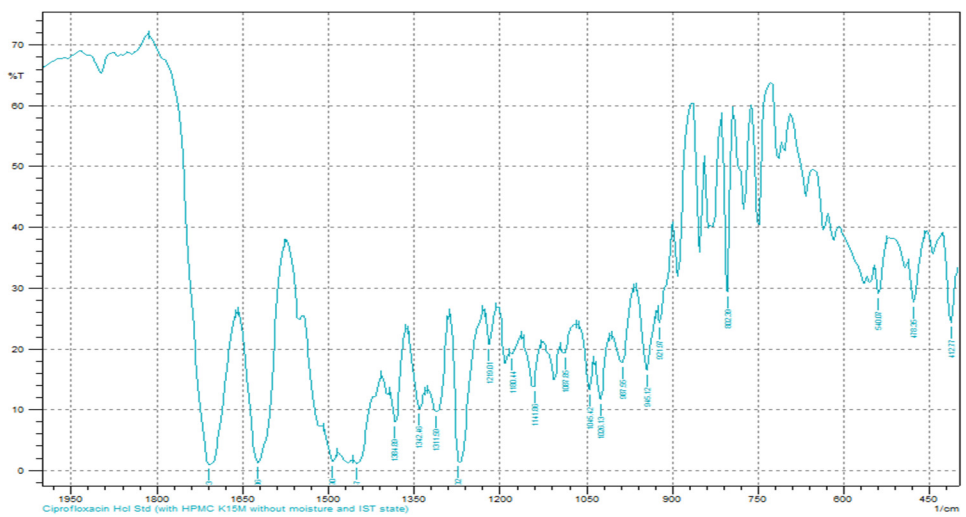

Figure 1(c): FTIR spectrum of ciprofloxacin HCl–HPMCK15M composite without moisture.

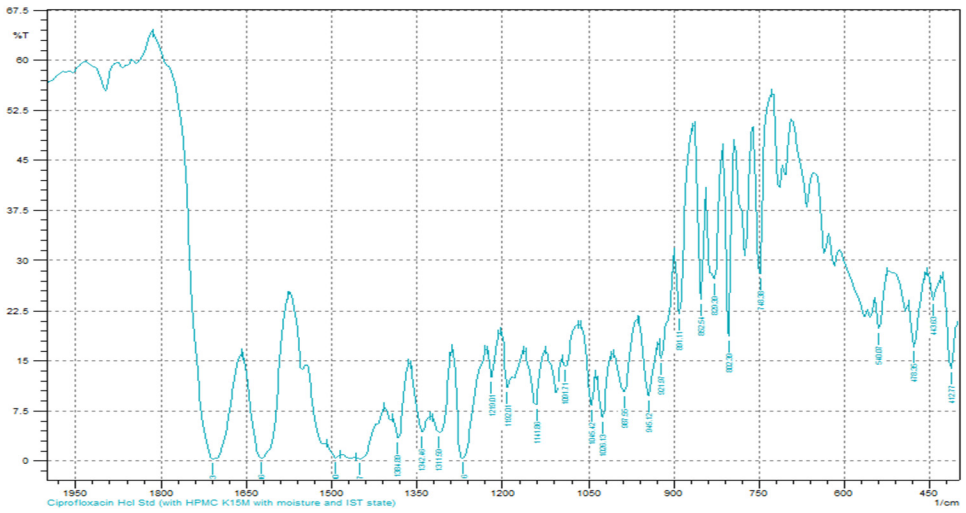

Figure 1(d): FTIR spectrum of ciprofloxacin HCl-HPMCK15M composite with 10% moisture under IST condition.

The FTIR spectrum of refrigerated ciprofloxacin-Carbopol 934p exhibited absorbance bands in the range of 412.77, 478.35, 540.07, 802.39, 852.54, 891.11, 921.97, 945.12, 987.55, 1026.13, 1045.42, 1145.72, 1192.01, 1219.01, 1269.16, 1311.59, 1342.46, 1384.89, 1450.47, 1492.9, 1624.06 and 1701.22 cm^{-1}. Similarly, FTIR spectrum of unimolar mixture of ciprofloxacin–Carbopol 934p with addition of 10% moisture under IST condition exhibited absorbance bands in the range of 412.77, 802.39, 829.39, 852.54, 891.11, 921.97, 945.12, 987.55, 1026.13, 1145.72, 1192.01, 1219.01, 1273.02, 1307.74, 1346.46, 1384.89, 1450.47, 1492.9, 1624.06 and 1708.93 cm^{-1} [Figures 1(e) and 1(f)].

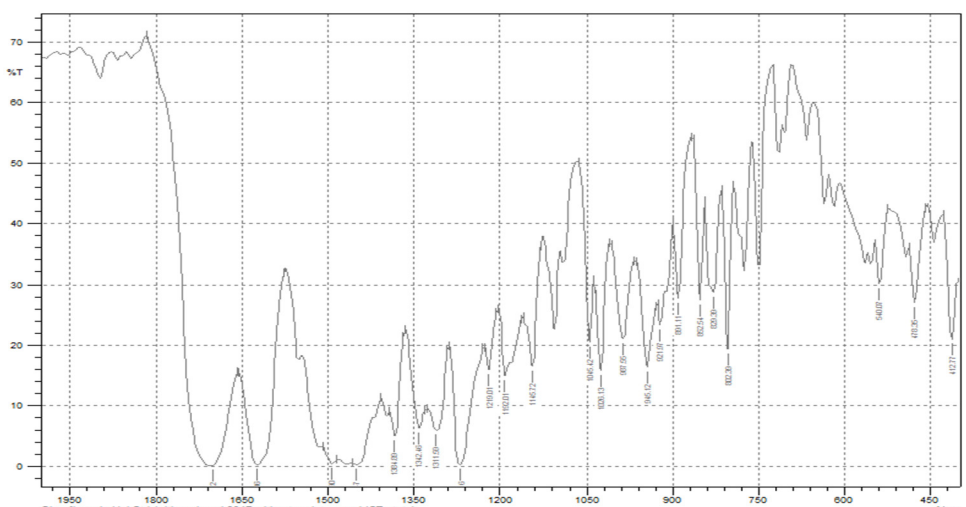

Figure 1(e): FTIR spectrum of ciprofloxacin HCl-carbopol 934p composite without moisture.

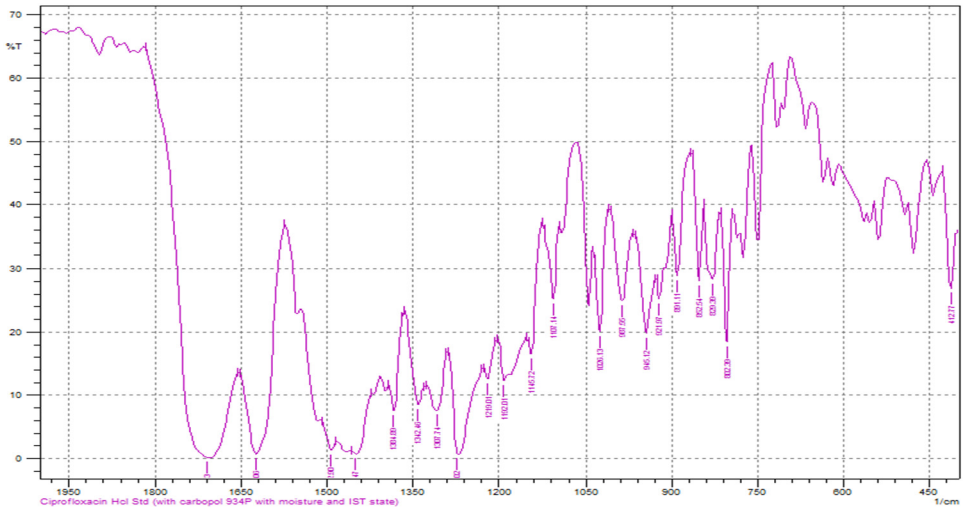

Figure 1(f): FTIR spectrum of ciprofloxacin HCl-carbopol 934p composite with 10% moisture under IST condition.

In the FTIR spectra of ciprofloxacin, one prominent characteristic absorbance band was found in between 1750 cm^{-1} and 1700 cm^{-1} representing carbonyl C=O stretching, i.e. C=O while the peak at 1650 cm^{-1} to 1600 cm^{-1} was assigned to quinolones. The bands at the 1450 cm^{-1} to 1400 cm^{-1} represented U_{C-O} and the ones at 1300 cm^{-1} to 1250 cm^{-1} suggested bending vibration of O-H group which indicated the presence of carboxylic acid. In addition, a strong absorption peak between 1050 cm^{-1} and 1000 cm^{-1} was assigned to C-F group.

Similarly, FTIR spectra of Carbopol 934p which showed the prominent peak between 1750 cm^{-1} and 1700 cm^{-1} was assigned to carbonyl C=O stretching band i.e. C=O while the peak at 1450 cm^{-1} to 1400 cm^{-1} was assigned to U_{C-O}/δ O-H. The band at 1250 cm^{-1} to 1200 cm^{-1} was assigned to U_{C-O-C} of acrylates. The ethereal cross linking is indicated by the prominent peak at 1160 cm^{-1}, represented a stretching vibration of U_{C-O-C} group. The band between 850 cm^{-1} and 800 cm^{-1} indicated out of plane bending of C=CH, i.e. δ=C-H. The absorbance band of ciprofloxacin–Carbopol 934p at 1650 cm^{-1} to 1600 cm^{-1} was assigned to O-C=O i.e. carbonyl asymmetric stretching vibration. A prominent peak at 1450 cm^{-1} was for O-C-O group of symmetric stretching vibration. The band from 1300 cm^{-1} to 1250 cm^{-1} was assigned to C-O-C stretching vibration of acrylates and esters. The peak between 1100 cm^{-1} and 1000 cm^{-1} represented C-F groups while the band at 800 cm^{-1} indicated the meta distribution of $\delta\text{-}_{Ar-H}$ group. Ciprofloxacin/EDTA composite was shown with their absorbance peak band within a wave number range between 2000 cm^{-1} and 400 cm^{-1} [Figure 1(g)].

In the FTIR spectra of the composites, it is obvious that the band position of C=O group was affected by esterification. Furthermore, conjugation involving C=O group lowering the C=O frequency between 1650 cm^{-1} and 1600 cm^{-1} might be due to the formation of β-ketoesters. The FTIR peaks assigned to U_{C-O} and U_{C-O-C} representing acrylates and esters confirm the esterification between polymeric OH group and –COOH

group of the drug (ciprofloxacin). The stretching vibration of C-F group remained nearly unaltered. The bending vibration of O-H group gives medium to strong bands in the region around 1450 cm^{-1}. The FTIR peak at 800 cm^{-1} gives the probability of out of plane bending of –ene bond and m-substitution of $\delta_{Ar\text{-}H}$ hydrogen atom. Both spectra showed prominent peaks for the stretching vibration of O-C-O and C=O groups, which prove the formation of esters between the drug and polymer. Both intermolecular and polymeric hydrogen bonding are also evident from the spectra of the polymeric composites. Thus, it may be predicted from FTIR analysis that the increases in atomic densities in the particular plane as well as the small change in orientation of the crystal lattice of the composites were due to formation of the esters and intermolecular hydrogen bonding between the carboxylic group of ciprofloxacin and carbopol polymers. FTIR analyses indicated that although there are intermolecular hydrogen bondings and esterification between ciprofloxacin and Carbopol 934p, ciprofloxacin retained its crystallinity in carbopol composites. The purity index of each composite system was observed above 0.95 with reference control standard (Table 5) and overlapping the FTIR spectra with each other suggested towards correlation between them (Nihar, Lila and Sujata 2011).

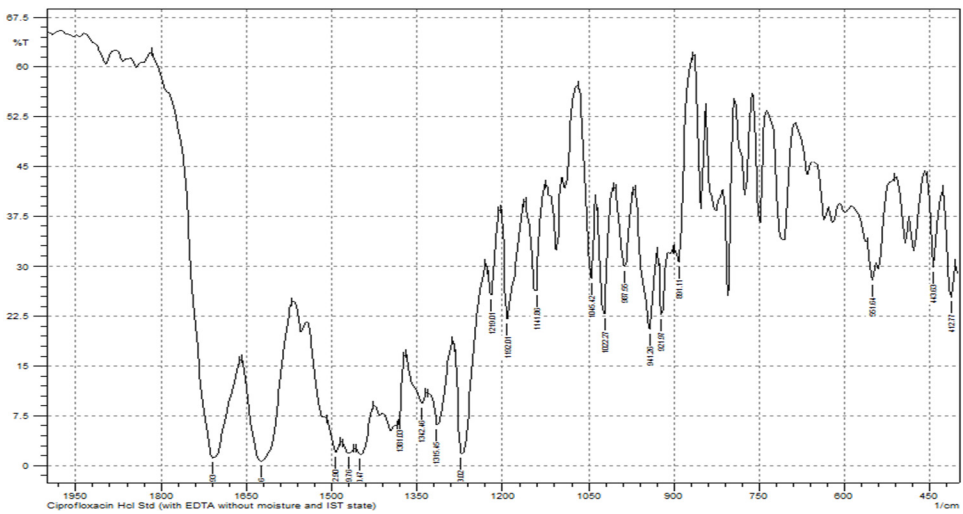

Figure 1(g): FTIR spectrum of ciprofloxacin HCl-EDTA composite without moisture.

Pre-treatment of Drug

Ciprofloxacin HCl exhibited instantaneous incompatibility with Carbopol 934p due to its acidic nature which turns the solution towards lower pH range, resulting in a lumpy precipitate. Drug entrapment within a resin system has been evaluated spectrophotometrically at different time interval based on Equation 1. The amount of drug loading was found higher in Indion®254 than Indion®214 at resin: drug equals 1:1 (91.84%) and 2:1 (95.30%). Based on drug loading efficiency, Indion®254 was selected. The optimised resin (Indion®254):drug was found to be 2.1:1.

FTIR Study of Resinates

The FTIR spectra of resinate (Indion®254: ciprofloxacin HCl = 2:1) and ciprofloxacin HCl exhibited similar absorbance bands, peak height, correction height, base height, base length, area and the corrected areas (Table 6). Similarly peak purity index of resinate (drug: resin; 1:2) was found to be 0.99 with overlapping of its peculiar functional groups with reference to standard ciprofloxacin HCl which suggest complete complexation phenomenon within binary mixture [Figure 1(h)].

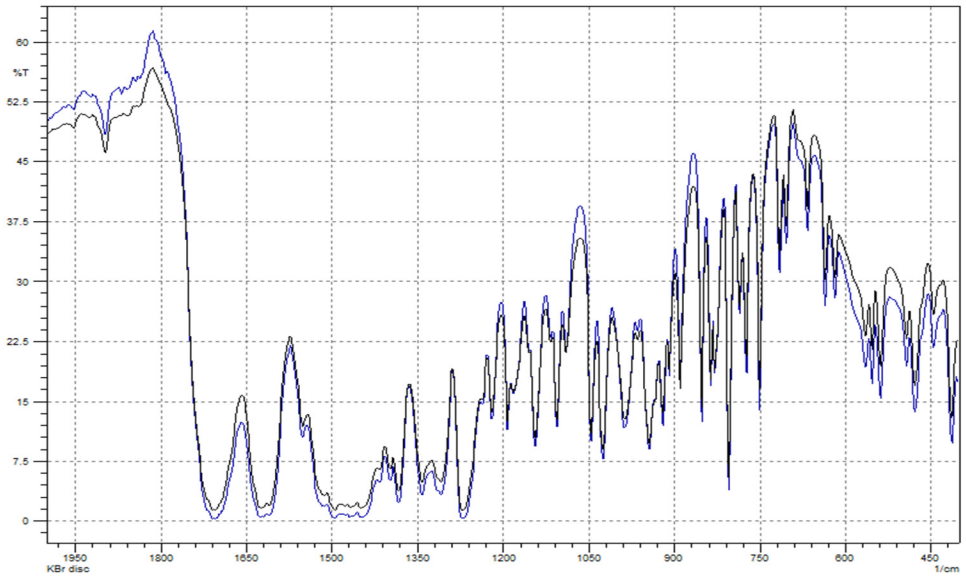

Figure 1(h): FTIR spectrum of ciprofloxacin HCl standard versus [ciprofloxacin HCl-Indion®254] complexation (resinate) at 1:2.

Physicochemical Characterisation

The physiochemical characterisation of *in situ* gelling system was carried out based on pH and *in situ* gelling capacity. The pH of the 13 formulations of central composite design was observed within range of 4.44 to 4.55.

Similarly, the *in situ* gelling capacity of the formulations was observed in a thermostatically maintained ATF in a vial. Out of the 13 formulations, F1, F6 and F11 had shown strong gel forming capacity. The gel formation was observed within a minutes and it remained for more than eight hours. F3, F4, F5, F8, F10, F12, and F13 had shown gel formation after few minutes and remained for 6–8 hours and F2 and F7 had formed gel after few minutes and the developed gel was dissolved within one hour. F2 had no gel forming capacity.

The drug content in developed and optimised formulation was found from 96.82% to 104.45%. The assay values of all the formulations are given in Table 4.

Table 4: Formulation design (Minitab 16) and their assay values.

	Carbopol 934p	HPMCK15M	Ciprofloxacin: Resin (Indion®254)	BKC	EDTA	Sodium acetate	Glacial acetic acid	Purified water qs	pH	Assay (%; *n* = 3)
	(% w/w)	(% w/w)	[1:2.0986]	(% w/v)	(% w/v)	(% w/v)	(ml)	(ml)		
F1	0.54	0.2	0.7303	0.01	0.05	0.135	0.075	100	4.51	96.82
F2	0.3	0.1	0.7303	0.01	0.05	0.135	0.075	100	4.53	102.92
F3	0.4	0.06	0.7303	0.01	0.05	0.135	0.075	100	4.55	103.12
F4	0.4	0.2	0.7303	0.01	0.05	0.135	0.075	100	4.48	103.53
F5	0.4	0.34	0.7303	0.01	0.05	0.135	0.075	100	4.45	102.20
F6	0.5	0.1	0.7303	0.01	0.05	0.135	0.075	100	4.50	103.20
F7	0.3	0.3	0.7303	0.01	0.05	0.135	0.075	100	4.47	104.45
F8	0.4	0.2	0.7303	0.01	0.05	0.135	0.075	100	4.50	103.38
F9	0.26	0.2	0.7303	0.01	0.05	0.135	0.075	100	4.54	101.35
F10	0.4	0.2	0.7303	0.01	0.05	0.135	0.075	100	4.44	100.60
F11	0.5	0.3	0.7303	0.01	0.05	0.135	0.075	100	4.48	101.31
F12	0.4	0.2	0.7303	0.01	0.05	0.135	0.075	100	4.53	99.87
F13	0.4	0.2	0.7303	0.01	0.05	0.135	0.075	100	4.51	101.30
$F_{optimized}$	0.3860	01889	0.7303	0.01	0.05	0.135	0.075	100	4.52	100.05

Notes: pH of the final system was adjusted to 4.50 ± 0.2 with glacial acid and 0.5 M NaOH.

Rheological Study

The viscosity of developed formulations at pH 4.5 ± 0.2 (before gel formation) and at higher pH range (pH ≥ 7.40) were evaluated with using spindle S64. The sol and gel phase of all the formulations showed shear thinning and pseudoplastic flow behaviour, which is desirable for ophthalmic formulations in order to prolong contact time with corneal membrane. The administration of ophthalmic preparations should have minimum influence over pseudoplastic character of pre-corneal tear film. As ocular shear rate is very large ranging from 0.03^{s-1} during interblinking to 4250^{s-1}–28500^{s-1} during blinking, viscoelastic fluids with a higher viscosity at low shear rate and low under high shear rate are desirable. All the formulations were found to be in liquid phase at pH 4.5 ± 0.2. Increase in the viscosity was observed when the pH of the formulations was raised to 7.4 which transform into gel phase. Similarly, the viscosity (cps) reading was decreased when angular velocity (shear rate) increased in both sol and gel phase and at higher angular velocities, viscosities are nearly constant at both pH state of system (Figure 2).

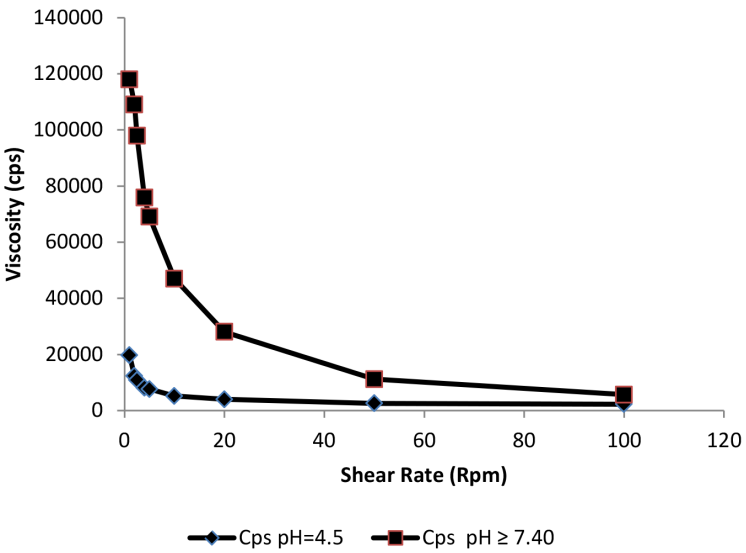

Figure 2: Rheological study of optimised formulation.

***In vitro* Release Study**

Cumulative percentage drug release versus time of 13 formulations is depicted in Figure 3. F9 and F5 showed the fastest and the slowest drug release rate.

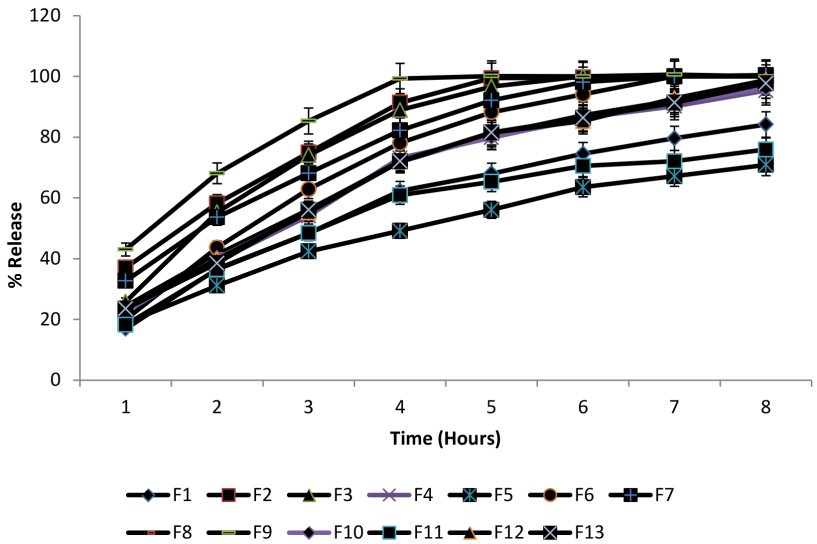

Figure 3: *In vitro* drug release profile of formulation F1–F13.

Optimisation of *In situ* Gelling Formulation

Optimisation of independent variables viz. Carbopol 934p and HPMCK15M in *in situ* gelling formulation was done in response to cumulative percentage drug release of 25, 75 and 98.66 at 1st, 4th and 8th hours, respectively. Contour plot [Figures 4(a), 4(b) and 4(c) and response surface (Figures 5(a), 5(b) and 5(c)] suggested 0.3860% (w/w) of Carbopol 934p and 01889% (w/w) of HPMCK15M.

Carbopol 934p is tightly coiled acidic molecules. Once dispersed in water, the molecules begin to hydrate and loose its coil structure. The polymers achieve maximum thickening behaviour upon conversion to a salt. Such behaviour can easily achieved by neutralising the polymers towards higher pH ranges with the use of common bases like NaOH or by other neutraliser. As the polymer has acidic behaviour having pH in the range of 2.5–3.5, it turns the pH of the solution towards lower range. As the pH of ciprofloxacin HCl is in the range of 3.5–4.5, the acidic carboxylic moieties of the polymer further lower the pH. As drug beyond its range may generate lumpy mass, therefore during addition of polymer the buffer system should be properly adjusted to avoid pH decrease. The instantaneous lumpy mass has been avoided by pre-treatment of drug prior addition. The modification has been carried out by entrapment of Indion®254 which is strongly cationic. The release of drug from resinate is based on ion-drug complex dissociation in presence of counter ion. The amount of resin in resinate also plays a pivotal role for drug release, as it plays rate limiting action. Similarly, the neutralising capacity of carbopol based formulation basically depends upon its concentration. The ratio of neutralising agent with carbopol should be considered during formulation. The non-neutralised dispersions of carbopol have very lower viscosity at a range of 4.5 and higher at neutral pH. As tear fluid has pH 7.40 which is neutral even itself, the triggering of physiochemical parameters phase transformation takes place from sol-gel by pH sensitive polymers like Carbopol 934p. Besides viscoelastic behaviour of polymer and phase transition feature, the release rate from drug-gelling matrix system should be considered. As Carbopol 934p has dominantly gelling property, the developed gel

gradually gets dissolved in tear fluid. The addition of hydrophilic polymer like HPMCK15M acts as release retardant. Similarly, considering upon weak buffering capacity of tear fluid and slightly acidic pH (4.50 ± 0.2), other additives should be added in formulation in order to mask ocular discomfort. The added HPMCK15M also minimises the pH based irritation with its lubricating action within *cul-de-sac* region.

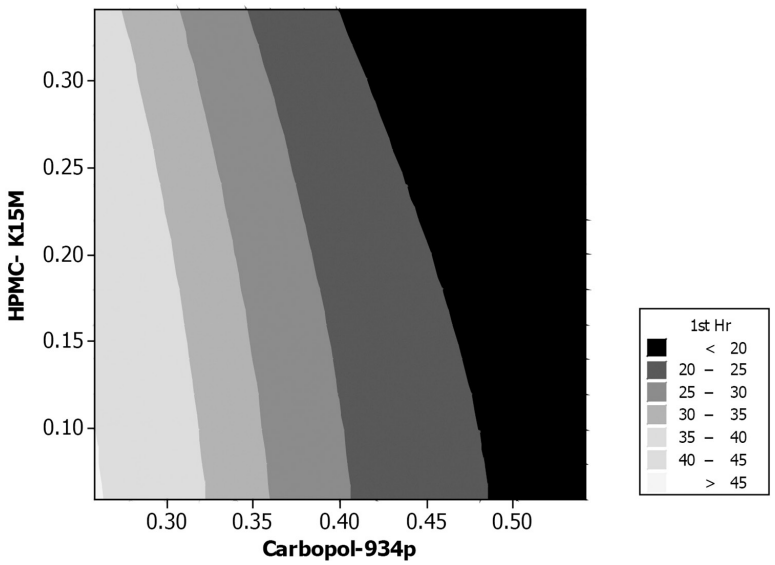

Figure 4(a): Counter plot at 1st hour drug release from *in situ* gelling system.

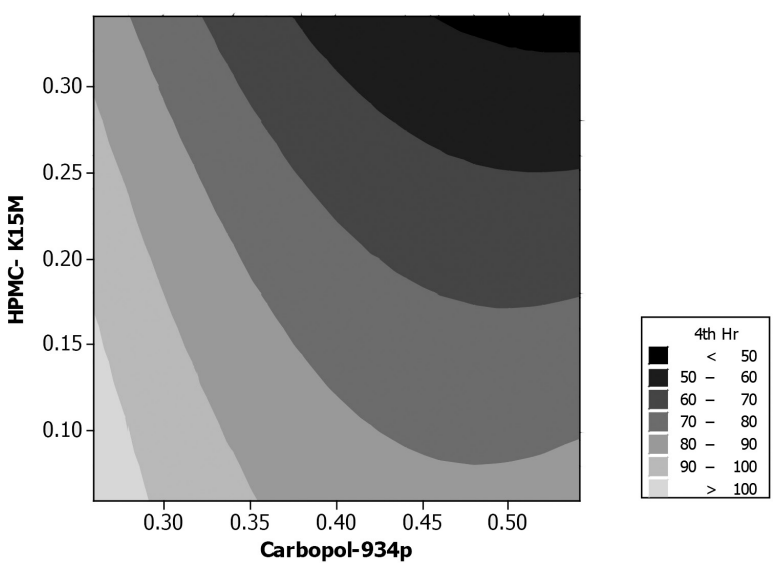

Figure 4(b): Counter plot of 4th hour drug release from *in situ* gelling system.

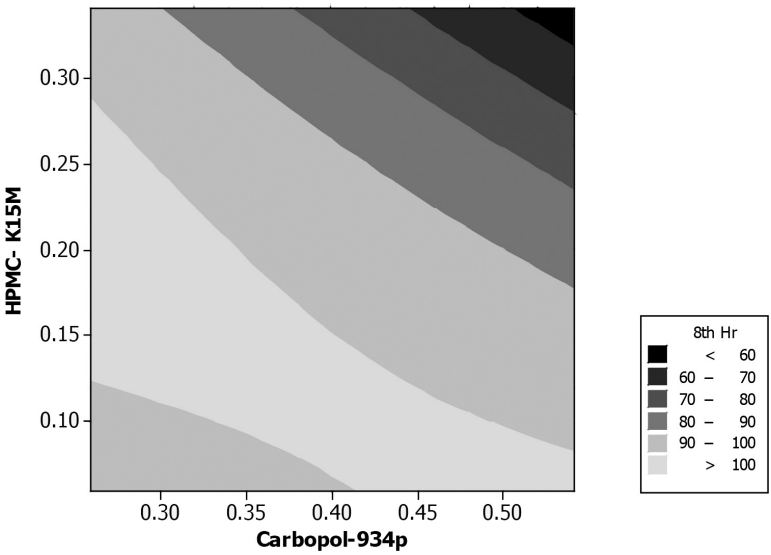

Figure 4(c): Counter plot at 8th hour drug release from *in situ* gelling system.

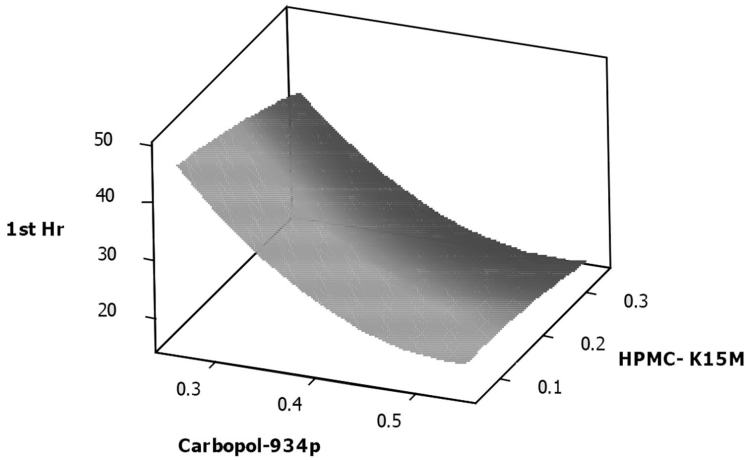

Figure 5(a): Surface response plot of 1st hour drug release from *in situ* gelling system.

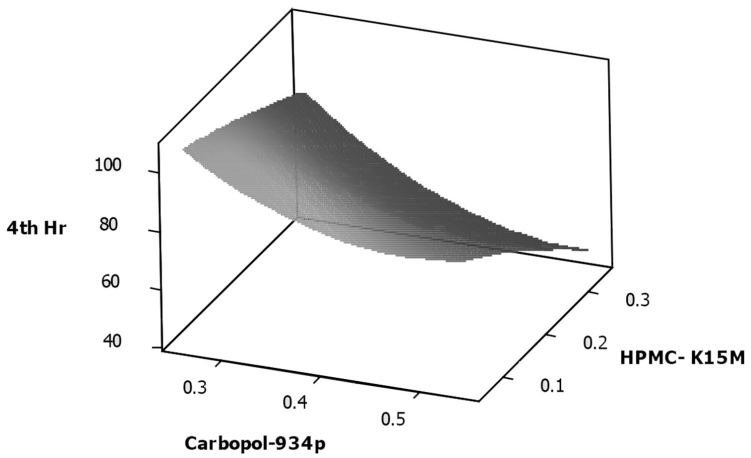

Figure 5(b): Surface response plot at 4th hour drug release from *in situ* gelling system.

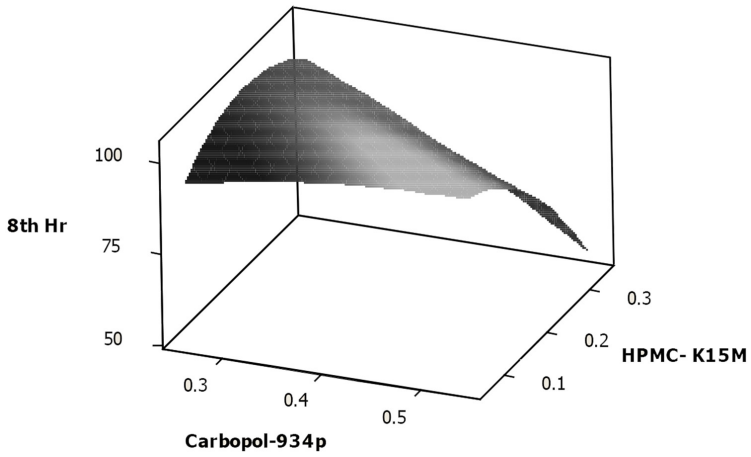

Figure 5(c): Surface plot at 8th hour drug release from *in situ* gelling system.

Ocular Irritancy Test

$F_{optimized}$ had shown mild degree of irritation having average score 0.33, 0.66 and 0.66 at 6th, 7th and 8th hour, respectively in HET-CAM technique. Since there was no high degree of irritation for a period of 8 hours, it has ocular comfort during delivery. The scoring pattern and obtained score of irritancy is depicted in Tables 5 and 6.

Table 5: Scoring scheme of ocular irritancy study by HET-CAM technique.

	Effect	Score	Inference
1.	No visible hemorrhage	0	Non-irritant
2.	Just visible membrane discoloration	1	Mild irritant
3.	Structures are covered partially due to membrane discolouration or hemorrhage	2	Moderate irritant
4.	Structures are covered totally due to membrane discolouration or hemorrhages	3	Severe irritant

Table 6: The ocular irritancy test of *in situ* gelling system by HET-CAM test.

	Formulation (time hours)		0	0.5	1	2	3	4	5	6	7	8
1.	Normal saline (0.9% w/v NaCl) (negative control)	Egg1	0	0	0	0	0	0	0	0	0	0
		Egg2	0	0	0	0	0	0	0	0	0	0
		Egg3	0	0	0	0	0	0	0	0	0	0
		Mean	0	0	0	0	0	0	0	0	0	0
2.	$F_{optimized}$	Egg1	0	0	0	0	0	0	0	0	0	0
		Egg2	0	0	0	0	0	0	0	0	1	1
		Egg3	0	0	0	0	0	0	0	1	1	1
		Mean	0	0	0	0	0	0	0	0.33	0.66	0.66
3.	Dioctyl sodium sulfosuccinate (1% w/w) (positive control)	Egg1	0	2	3	3	3	3	3	3	3	3
		Egg2	0	2	3	3	3	3	3	3	3	3
		Egg3	0	2	3	3	3	3	3	3	3	3
		Mean	0	2	3	3	3	3	3	3	3	3

Antimicrobial Efficacy Test

The antimicrobial efficacy of $F_{optimized}$ was carried out with reference to marketed sample (Microflox) based on zone of inhibition technique for *Pseudomonas aeruginosa* (ATCC 10145) and *Staphylococcus aureus* (ATCC 25903). The test was taken at a concentration of 3 µg/ml, 30 µg/ml, 90 µg/ml and 300 µg/ml. $F_{optimized}$ had no inhibitory effect at 3 µg/ml for both microorganisms. At 30 µg/ml, 90 µg/ml and 300 µg/ml, $F_{optimized}$ had 100%, 95.23% and 96% towards *Staphylococcus aureus* (ATCC 25903) and 93.75%, 100% and 96% towards *Pseudomonas aeruginosa* (ATCC 10145).

Regression Equations for Drug Release Prediction

Equation derived from the regression coefficients of drug release at 1st, 4th and 8th hour are given in Equations (12), (13) and (14), respectively:

Regression equation for 1st hour:

$$Y = 120.37 - 373.71X_1 - 30.12X_2 + 344.18X_1^2 - 39.08X_2^2 + 64.25X_1X_2 \quad (12)$$

Regression equation for 4th hour:

$$Y = 197.71 - 472.19X_1 + 10.37X_2 + 504.89X_1^2 - 82.36X_2^2 - 201.50X_1X_2 \quad (13)$$

Regression equation for 8th hour:

$$Y = 50.91 + 158.55X_1 + 343.32X_2 - 118.56X_1^2 - 449.81X_2^2 - 611.50 \quad (14)$$

where, X_1 = Carbopol 934p

X_2 = HPMCK15M

Drug Release Kinetics

Out of 13 formulations, F1, F3, F4, F5, F6, F9 and F11 followed first order kinetics, F8 and F10 followed Higuchi model and F2, F7, F9, F12 and $F_{optimized}$ followed Krosmeyer-Pepas as model. Carbopol 934p showed dominant effect against drug release over its gelling capacity. Swelling of Carbopol 934p in ATF could be due to neutral pH environment. Carbopol 934p causes ionic repulsion of polymer due to presence of electrolyte in tear fluid which is manifested on the macro level as swelling (Durrani *et al*. 1994). The hydration of polymer resulted in rapid decrease in its glass transition temperature to the temperature of the medium.

Microscopically, there is a relaxation response of the polymer chains due to stresses introduced by the presence of the dissolution medium. This results in an increase in the radius of gyration and end-to-end distances of the polymer chains, causing a significant increase in the molecular volume of the hydrated polymer (Ranga-Rao and Devi 1988). This reduces the free volume due to presence of the micropores, which may manifest itself as a shift in the drug release mechanism. Increasing the amount of Carbopol 934p in the formulations resulted in reduction in the drug release rate with swelling controlled mechanism.

Similarly, HPMCK15M takes up water and polymer starts hydrating to form a gel layer. An initial burst of soluble drug may occur due to surface leaching when a composite containing a swellable glassy polymer comes in contact with an aqueous medium. There is an abrupt change from a glassy to a rubbery state which is associated with swelling process with time and water infiltrated deep into the case increasing the thickness by the gel layer. After its hydration, it follows states of dissolving or eroding. When water reaches the center of the system and the concentration of drug falls below the solubility value, the release rate of drug begins to reduce. At the same time, an increase in thickness of the barrier layer with time increases the diffusion path length, reducing the rate of drug release. Drug release kinetic associated with these gels layer dynamic, range initially from Fickian to anomalous (non-Fickian). In general, two major factors control the drug release from swelling controlled system by a relaxation process (hydration, gelatin or swelling) followed by erosion. The optimised formulation followed Korsmeyer-Peppas equation (R^2 = 0.985) and diffusion exponent 0.874 that indicate non-Fickian diffusion in gelling system. As a result of these simultaneous processes, two front are evident, a swelling front, where the polymer get hydrated and an eroding front. The distance between these two fronts are called diffusion layer thickness. Diffusion layer thickness depends on the selective rate at which the swelling and eroding fronts move in relation to each other.

Similarity and Dissimilarity Factor

The similarity and dissimilarity study of drug release was carried out between predicted values obtained by using regression equations 12, 13 and 14 and observed values. Similarity factor F_s (87.01) and the dissimilarity factor F_d (1.81) showed there is similarity between observed and predicted values.

Drug Release Comparison of Conventional Marketed Eye-Drop (Microflox) with $F_{optimized}$ *in situ* Gelling System

The drug release of optimised *in situ* gelling formulation was compared with Microflox purchased from retail pharmacy. The *in vitro* drug release study was carried out in modified dissolution apparatus through cellophane membrane. The cumulative percentage drug release of $F_{optimized}$ and eye drop versus time (minute) plot (Figure 6) showed that Microflox took two hours to release the total drug but $F_{optimized}$ took 8 hours as Microflox did not contain rate retarding polymers.

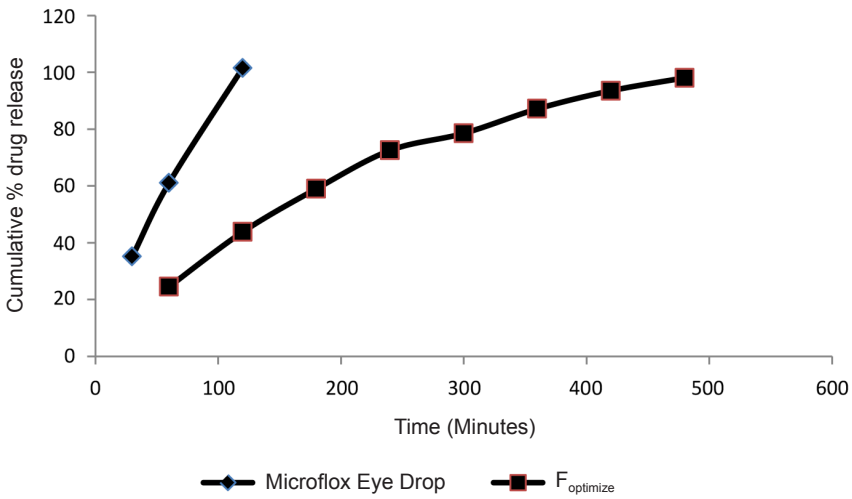

Figure 6: Comparison of *in vitro* release of $F_{optimized}$ *in situ* gelling system with conventional eye-drop (Microflox).

CONCLUSION

A significant challenge to the formulator in ophthalmic drug delivery is to circumvent the protective barriers of the eye without causing permanent tissue damage. Due to unique physiological anatomy of eye and its underlying barriers, ophthalmic drug delivery system still seems to be challenging. The lower residence contact time of drug, higher tear turnover, limited area of contact and impermeability of corneal epithelium layer make lower bioavailability of conventional dosage form. Phase changing polymer can help to overcome these limitations to enhance bioavailability. This study indicates use of Carbopol 934P and HPMCK15M for *in situ* gelling formulation of ciprofloxacin HCl.

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