Journal of Pharmaceutics & Drug Development
Volume 7 | Issue 1
ISSN: 2348-9782

Research Article Open Access

Formulation and Evaluation of Sustained Occular Delivery of Ciprofloxacin Hydrochloride

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Citation: Maharjan S, Pradhan A, Shrestha M, Koju S (2019) Formulation and Evaluation of Sustained Occular Delivery of Ciprofloxacin Hydrochloride. J Pharma Drug Develop 7(1): 103

Received Date: July 21, 2020 **Accepted Date:** August 24, 2020 **Published Date:** August 26, 2020

Abstract

The aim of the present work was formulation and evaluation of in-situ gelling system of Ciprofloxacin Hydrochloride. The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solution due to rapid precorneal elimination of the drug may be overcomed by the use of in-situ gel forming system that are installed as drop into the eye and undergo sol to gel transition in the cul-de-sac. Hence, the purpose of the present work was to formulate ion activated in-situ gelling system of Ciprofloxacin Hydrochloride to provide sustained release of drug based on polymeric carriers. Thirteen formulations of 0.4% Ciprofloxacin ophthalmic in situ gel were formulated using various concentration of Sodium Alginate (0.2% to 0.5%) and Hydroxy propyl methyl cellulose (HPMC K100) (0.1% to 0.7%) using Minitab. pH of the formulations was adjusted within 6.5±0.1 in order to achieve maximum solubility of drug as well as to avoid ocular irritation after instillation in cul de sac. The prepared in situ gels were then evaluated for visual appearance, clarity, pH, drug content, gelling capacity, rheological studies, sterility testing, and in vitro drug release studies. The developed formulations was clear, efficacious, stable, and provided sustained release over 8-hour.

Keywords: Ciprofloxacin Hydrochloride; In-Situ Gel; Sodium Alginate; PH; Sustained Release

Introduction

The conventional liquid ophthalmic formulation is eliminated from the pre corneal area immediately upon instillation because of lacrimal secretion and nasolacrimal drainage. Only 10% drug concentrations is available at the site of actions. Some conventional ophthalmic preparation such as gels, ointment, and viscous preparation were reported to blurred vision and these preparations have no bio adhesive property [1].

In-situ gelling system has become one of the most prominent among novel drug delivery systems due to many advantages such as improved patient compliance, reduced frequency of drug administration. There are many triggering mechanisms in in-situ gel formation. Some of them are pH change, temperature modification and solvent exchange. In-situ gels are the formulations that are in sol form before administration in the body, but once administration undergo gelation to form gel. Various routes of administration of in-situ gelling systems are oral, nasal, ophthalmic, vaginal, injectable, intraperitoneal and rectal route [2].

Depending on the method employed to cause sol to gel phase transition on the ocular surface, the following three types of systems have been recognized:

pH-triggered: The polymers used in this system are pseudo latexes - carbomer (carbopol), cellulose acetate phthalate latex (CAP-latex).

Temperature-dependent: The polymers used in this system are Poloxamers (Pluronic), cellulose derivatives (MC, HPMC), Xyloglucan.

Ion-activated: The polymers used in this system are Alginates, Gelrite

The polymers chosen to prepare ophthalmic in situ gels should meet some specific rheological characteristics. It should always be noted that the instillation of a formulation should influence tear behaviour as little as possible. Newtonian formulations have a constant viscosity independent of the shear rate, whereas pseudo plastic solution exhibit decreased viscosity with increasing shear rate. In situ gels are formulated in such a way that they show Newtonian behaviour before administration to eye, whereas shows pseudo plastic behaviour upon instillation in eye, offering lowered viscosity during blinking and stability of the tear film during fixation [3].

In ion activated gelling system, solution is triggered by cations present in eye tear fluid like Na+, Ca++ and Mg++. Generally anionic polymers are used in the formation of ion sensitive drug delivery system. Polymers like sodium alginate, gelrite, tamarind gum, gellen gum are used in these formulations. Various other polymers like methylcellulose (MC), hydroxyl propyl methyl cellulose (HPMC) are used in combination of these polymers to increase the effect. They provide sustained release of drug by providing mucoadhesiveness (Figure 1).

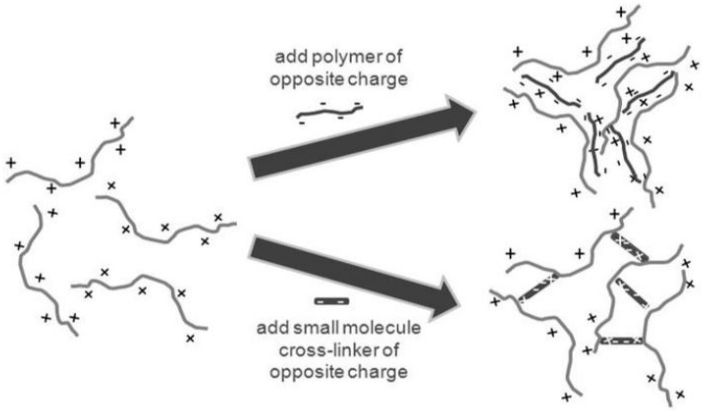

Figure 1: Mechanism showing ion activated system

Ion-activated polymers, most widely used in ophthalmic formulations, are gellan gum and sodium alginates (ALG). The interaction of the cations presents in the tear fluid and the negatively charged polysaccharide promotes a crosslinking structure which improves the residence time and the bioavailability of the formulation in the ocular globe.

Ciprofloxacin hydrochloride is a pale yellow, crystalline powder which contains Fluoroquinolone group. Ciprofloxacin hydrochloride is used as an antibacterial agent in the treatment of corneal ulcers caused by susceptible strains of bacteria, including *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia,* etc.

Ciprofloxacin's bactericidal action is due to interference with the enzyme DNA gyrase, which is needed for the synthesis of bacterial DNA. It inhibits this enzyme hence will not allow multiplication of bacterial cell [4].

Materials and Methods

Materials

List of raw materials used are shown in Table 1 below:

S.N.	Materials	Mfg By
1	Ciprofloxacin Hydrochloride	Hukam Pharmaceuticals Pvt Ltd.
2	Sodium Alginate	Himedia
3	Hypromellose	Colorcon
4	Tween 80	Fischer Scientific
5	Sodium Chloride	Fischer Scientific
6	Benzalkonium Chloride 50%	Fischer Scientific
7	Calcium Chloride Dihydrate	Himedia
8	Sodium Bicarbonate	Fischer Scientific
9	Potassium Dihydrogen Phosphate	Fischer Scientific
10	Di-sodium Hydrogen Phosphate	Fischer Scientific
11	Soyabean Casein Digest Agar	Himedia

Table 1: List of materials with their specification

Linearity

In order to demonstrate linearity, various concentrations of Ciprofloxacin Hydrochloride were prepared in phosphate buffer pH 6.5, i.e 3ppm, 4ppm, 5ppm, 6ppm,7ppm and 8ppm. All the concentrations were first scanned in UV range (200-400 nm) for determination of maximum wavelength (λ_{max}) which was found to be 272 nm as shown in Table 2.

Concentration (ppm)	Absorbance
3	0.240
4	0.346
5	0.445
6	0.540
7	0.649
8	0.744

Table 2: Various concentration of Ciprofloxacin HCl and their absorbance

Then corresponding absorbance values were scanned for all the concentrations in λ_{max} (272 nm). The absorbance versus concentration was plotted as in Figure 2 based on Table 2 in order to estimate correlation coefficient (R^2) and linearity equation. All the analytical works were performed in UV-Visible spectrophotometer, Shimadzu, UV-1800 model.

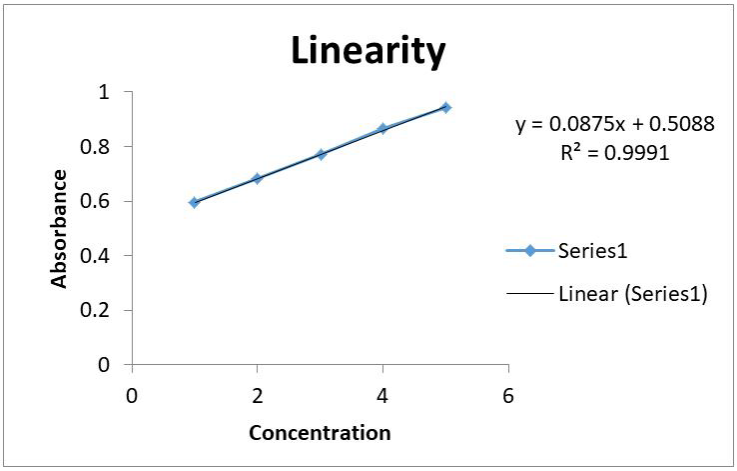

Figure 2: Calibration curve of Ciprofloxacin Hydrochloride

Selection of Buffer (Vehicle)

In order to ensure product stability and desired drug solubility for Ciprofloxacin Hydrochloride in-situ gelling system, various buffers like acetate buffer IP (pH 6.0), phosphate buffer IP (6.5, 6.8, 7.0), citrophosphate buffer IP (6.7, 7.2) were selected.

Preliminary Study

Preliminary study was carried out for selection of optimum concentrations for different polymers, by mixing different concentration of Polymers (Sodium alginate and HPMC K 100) in selected buffer (Phosphate buffer pH 6.5) and were evaluated for gelling capacity as shown in Table 3 below:

Batch code	Sodium Alginate (%)	HPMC (%)	Gelling Capacity
F1	0.1	0	no gelling
F2	0.1	0.1	no gelling
F3	0.1	0.3	no gelling
F4	0.1	0.4	no gelling
F5	0.1	0.5	+
F6	0.1	0.6	+
F7	0.1	0.7	+
F8	0.1	0.8	+
F9	0.2	0	no gelling
F10	0.2	0.1	+
F11	0.2	0.3	++
F12	0.2	0.4	++
F13	0.2	0.5	++
F14	0.2	0.6	++

Batch code	Sodium Alginate (%)	HPMC (%)	Gelling Capacity
F15	0.2	0.7	++
F16	0.2	0.8	++
F17	0.3	0	+
F18	0.3	0.1	+
F19	0.3	0.3	+
F20	0.3	0.4	++
F21	0.3	0.5	++
F22	0.3	0.6	+++
F23	0.3	0.7	+++
F24	0.3	0.8	++
F25	0.4	0	no gelling
F26	0.4	0.1	+
F27	0.4	0.3	+
F28	0.4	0.4	++
F29	0.4	0.5	++
F30	0.4	0.6	++
F31	0.4	0.7	++
F32	0.4	0.8	+++
F33	0.5	0	no gelling
F34	0.5	0.1	++
F35	0.5	0.3	++
F36	0.5	0.4	+++
F37	0.5	0.5	+++
F38	0.5	0.6	+++
F39	0.5	0.7	+++
F40	0.5	0.8	+++

Where, + indicates gelation occurred after a few minutes and gel dissolved rapidly,
++ indicates immediate gelation and remained up to 8 hours,
+++ indicates immediate gelation but remains more than 10 hours.
Table 3: Formulation of Preliminary Batches and their Evaluation

Methodology

Varying concentrations of sodium alginate and HPMC K100 were used to prepare the formulation. Various concentrations of HPMC K100 were dissolved in 70ml phosphate buffer 6.5 and stirred slowly with magnetic stirrer. Sodium alginate was sprinkled over the solution and allowed to hydrate overnight. The solution was again stirred with magnetic stirred after 24 hours.

0.444gm of Ciprofloxacin Hydrochloride was dissolved in phosphate buffer 6.5 separately and then drug solution was added to the above solution under constant stirring until a uniform solution was obtained. 0.9 gm NaCl, 1ml benzalkonium chloride and tween 80 were added. Then the volume was made up to the 100ml volume. The developed formulations were filled in suitable vials under aseptic condition, sterilized in the autoclave (121 ^{o}C and 15 p.s.i) for 20 minutes and further evaluations were carried out (Table 4) [5].

FN	Ciprofloxacin HCl (gm)	Sodium alginate (gm)	HPMC K 100 (gm)	Tween 80 (ml)	Benzylalkonium (ml)	Sodium Chloride (gm)	Phosphate buffer pH 6.5(q.s)
F1	0.444	0.500	0.700	1	0.002	0.9	100
F2	0.444	0.350	0.400	1	0.002	0.9	100
F3	0.444	0.350	0.024	1	0.002	0.9	100
F4	0.444	0.305	0.400	1	0.002	0.9	100
F5	0.444	0.200	0.100	1	0.002	0.9	100
F6	0.444	0.138	0.400	1	0.002	0.9	100
F7	0.444	0.350	0.400	1	0.002	0.9	100
F8	0.444	0.350	0.824	1	0.002	0.9	100
F9	0.444	0.350	0.400	1	0.002	0.9	100

FN	Ciprofloxacin HCl (gm)	Sodium alginate (gm)	HPMC K 100 (gm)	Tween 80 (ml)	Benzylalkonium (ml)	Sodium Chloride (gm)	Phosphate buffer pH 6.5(q.s)
F10	0.444	0.562	0.400	1	0.002	0.9	100
F11	0.444	0.500	0.100	1	0.002	0.9	100
F12	0.444	0.200	0.700	1	0.002	0.9	100
F13	0.444	0.350	0.400	1	0.002	0.9	100

Table 4: Formulation designed by Minitab 17
For the design of all the formulations and analysis of the results, Minitab 17 software was used.
The concentration range of polymers used in the formulation was as per preliminary study:
For Sodium alginate: Low: 0.2%, High: 0.5%
For HPMC: Low: 0.1%, High: 0.7%

Evaluation

Following evaluations were performed based upon availability of resources.

Appearance and Clarity

The appearance and clarity of all the formulations were determined visually against white and black background.

pH

The pH of all the formulations was measured by calibrated pH meter.

Gelling Capacity

The gelling ability of the prepared formulations was determined visually. The gelling capacity was determined by pouring a drop of the formulation in a vial containing 5ml artificial tear fluid which was freshly prepared and equilibrated at 37 °C, and both the time of gelation and the time taken for the gel formed to dissolve was noted (Table 5) [6].

NaCl	0.670g
NaHCO3	0.200g
$CaCl_2$	0.008 g
Purified Water	q. s. 100 g

Table 5: Composition of artificial tear fluid

Drug Content

The drug content of Ciprofloxacin hydrochloride in the in-situ gel was carried by using UV-Visible spectrophotometer. In order to estimate Ciprofloxacin content in formulation, 1gm of in-situ gel formulation was weighed and transferred into 100ml volumetric flask. About 70 ml of phosphate buffer was added and the solution was sonicated for about 15 minutes. Then the solution was filtered through whattman No.1 filter with discarding first few filtrates. 5 ml of the filtrate was further diluted to 50 ml with phosphate buffer in order to obtain the final concentration of about 5ppm. Standard of about same concentration was prepared in similar manner. Both standard and sample solutions were scanned for absorbance in UV-Visible Spectrometer at λmax of 272 nm using phosphate buffer as blank solution. The corresponding absorbance was noted.

Rheological Study

The viscosity of the formulation was determined using a Brookfield viscometer (BDV-8S). The developed formulation was poured into the small beaker. Spindle L1 was used and the angular velocity (shear rate) was increased gradually from 0.3 to 60 rpm. A typical run comprised changing angular velocity from 0.3 to 60 rpm with equal wait for each rpm. The angular velocity was reversed (from 60 to 0.3) with similar wait. The average of two readings was used to calculate the viscosity [7].

In Vitro Release Study

The in vitro release of Ciprofloxacin from the formulations was studied through cellophane membrane, using a modified dissolution testing apparatus. The dissolution medium used was freshly prepared artificial tear fluid (ATF) pH 7.4. Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to the base of basket as shown in Figure 3 below.

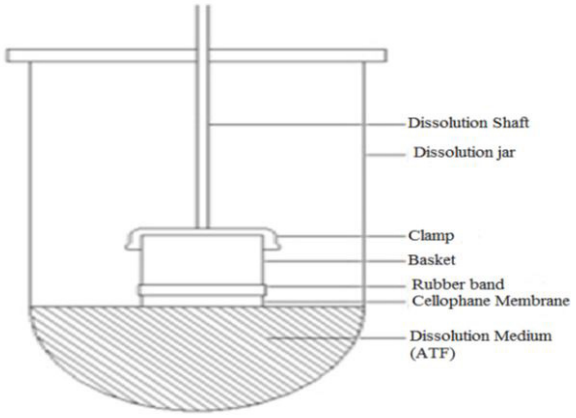

Figure 3: Arrangement of basket for in vitro drug release study [9]

Volume of 1 ml of the formulation was accurately pipetted into this assembly. The basket was attached to the metallic driveshaft and suspended in 100 ml of dissolution medium maintained at 37±1 °C so that the membrane just touched the dissolution medium surface as in Figure 2. The shaft was rotated at 50rpm [8].

Aliquots, each of 10ml volume, were withdrawn at hourly intervals and replaced by an equal volume of fresh ATF. The aliquots were filtered and 2 ml of the solution was further diluted to 10 ml in volumetric flask with ATF. The solutions were then analyzed by UV-Visible spectrophotometry at 272 nm [9].

Kinetic Study

The release kinetics of ciprofloxacin from in situ gel formulation was evaluated considering four different models including zero order, first order, Higuchi model, and Korsmeyer's peppas model.

Sterility Study

The test for sterility was carried out under aseptic conditions and all the experimental works were performed inside laminar air flow bench.

Soya-bean casein digest agar (SCDA) medium is suitable for the culture of aerobic bacteria. So, the media was selected for the experiment. After preparation of the media, it was sterilized by autoclaving at 121 °C for 20 minutes at 15 p.s.i.

Result and Discussion

Selection of Buffer

Based on results obtained from solubility data of Ciprofloxacin hydrochloride in various buffer, as the drug has maximum solubility in phosphate buffer pH 6.5, the solution of Ciprofloxacin hydrochloride in the buffer was further tested for stability to light, temperature and autoclaving. The colour of the formulation seemed to be slightly changed when exposed to light. It suggests the formulation to be packed and stored in light resistant container. Rise in temperature and autoclaving didn't show any significant changes in the drug-buffer solution. Hence, due to maximum solubility and stability of the drug Ciprofloxacin hydrochloride, phosphate buffer pH 6.5 was selected as vehicle for preparation of the formulations (Table 6).

Buffer	Solubility
Phosphate buffer pH 6.5	+
Phosphate buffer pH 6.8	-
Phosphate buffer pH 7.0	-
Citrophosphate buffer pH 6.0	-
Citrophosphate buffer pH 7.0	-
Citrophosphate buffer pH 7.2	-
Acetate buffer pH 6.0	-

Where, (+) indicates ciprofloxacin hydrochloride is soluble in buffer,
(-) indicates ciprofloxacin hydrochloride is not soluble in buffer.
Table 6: Solubility of Ciprofloxacin Hydrochloride with various buffers

Compatibility Study

IR spectroscopic studies were conducted to determine possible drug-polymer interactions. IR spectra of pure drug Ciprofloxacin Hydrochloride and polymers Sodium Alginate and HPMC K100 and their physical mixture in the ratio of 1:1 (Drug: Polymer), were observed. Ciprofloxacin Hydrochloride with Sodium Alginate and HPMC K100, when scanned in combination showed compatibility with standard Ciprofloxacin Hydrochloride as shown in Figure 4 and 5 below.

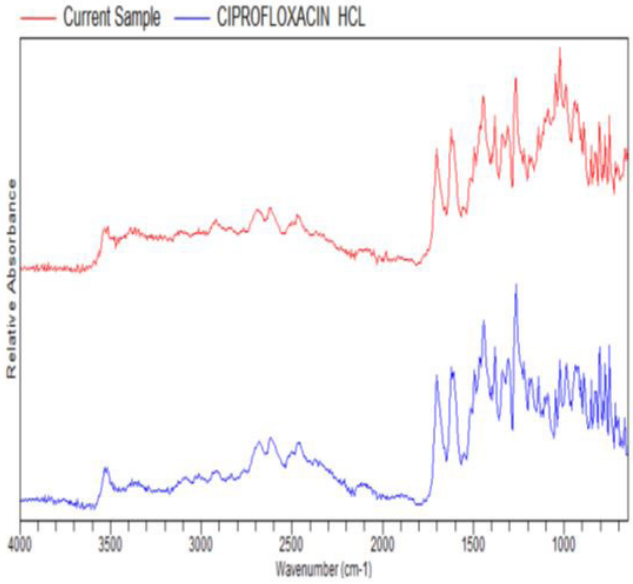

Figure 4: Interpretation of IR Spectra of Ciprofloxacin HCl and HPMC1K00

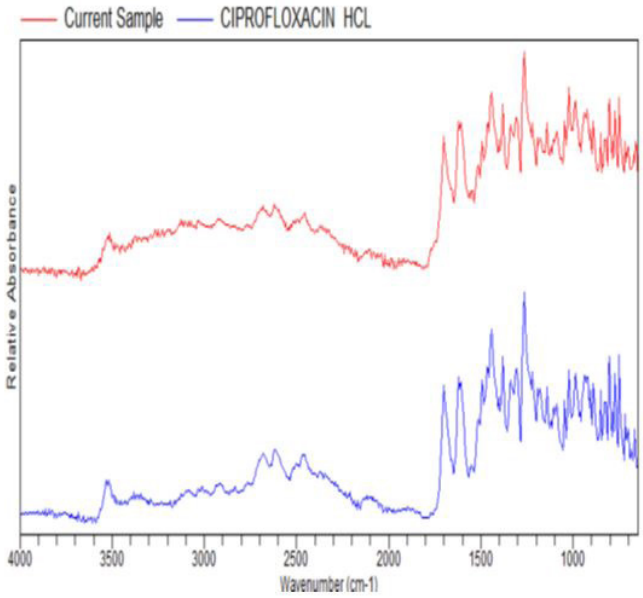

Figure 5: Interpretation of IR Spectra of Ciprofloxacin HCl and Sodium alginate

Appearance, pH, Gelling Capacity and Drug Content

When examined against white and dark background, the solutions were devoid of any gritty particles. All the formulations were clear except F1, F8 and F12 due to high concentration of polymer, which were slightly turbid. The pH values for all formulations were within acceptable range (3.5 to 10.5) and would not cause any irritation upon administration of the formulation.

In-situ gelling capacity of developed formulation was observed in a thermostatically maintained artificial tear fluid in a vial. Out of the thirteen formulations, formulations F1 and F10 have shown strong gel forming capacity. The gel formation was observed within a minute and it remained for more than 8 hours. The formulation F2, F4, F7, F8, F9, F11, F12 and F11 have shown gel formation after few minutes and remained for 6-8 hours. The formulations F3 and F5 have shown low gelling capacity. The gel was observed after few minutes and the developed gel was dissolved within one hour. The formulation F6 has shown no gel formation. The optimized formulation also have shown gel formation after few minutes and remained for 6-8 hours (Table 7).

FN	Clarity	pH	Gelling Capacity	Drug Content
F1	slightly turbid	5.1	+++	100.67
F2	Clear	5	++	95.59
F3	Clear	5.2	+	99.47
F4	Clear	5	++	96.54
F5	Clear	5	+	95.11
F6	Clear	5.1	no gelling	101.38
F7	Clear	5	++	98.45
F8	slightly turbid	5	++	100.02
F9	Clear	5	++	95.23
F10	Clear	5.2	+++	99.13
F11	Clear	5	++	99.82
F12	slightly turbid	5	++	98.85
F13	Clear	5	++	99.23
Optimized	Clear	5.3	++	100.2

Table 7: Clarity of optimized and all formulation

Rheological Study

The ophthalmic preparations should show Newtonian behaviour before administration so that they show good flow property for easy administration but should show Pseudo Plastic behaviour i.e. high viscosity under low shear and low viscosity under high shear, since the ocular shear rate is very large ranging from 0.03 s^{-1} during inter-blinking periods to 4250-28500 s^{-1} during blinking.

For the formulations prepared, on increasing the shear rate from 0.3 to 60 rpm, F3, F5, F11, and Optimized formulations showed Newtonian behaviour whereas other formulations showed Pseudo-plastic behaviour as shown in Figure 6.

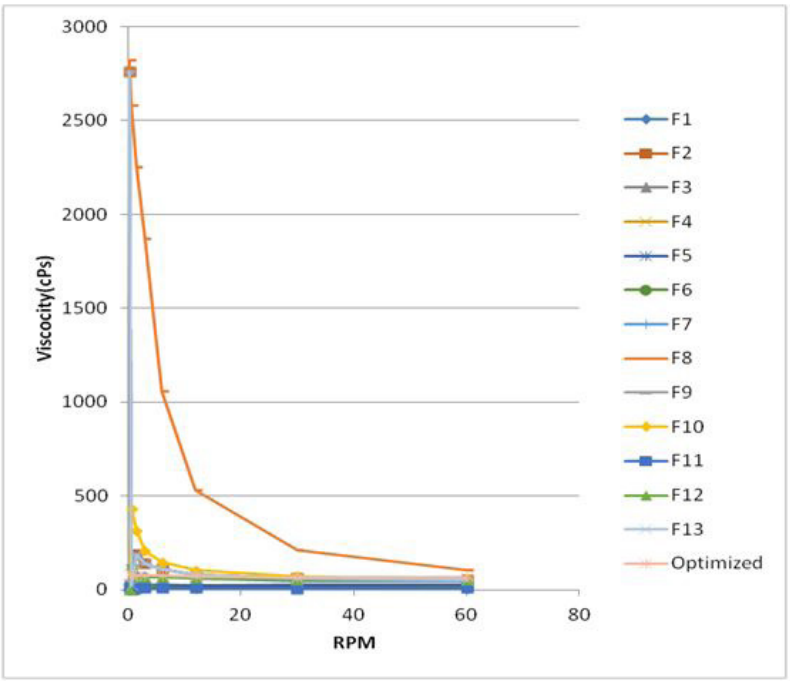

Figure 6: Viscosity of formulation (F1 to F13) and optimized formulation

So, F3, F5, F11 and Optimized formulations were subjected for further study at physiological conditions (in ATF and Temperature 37 °C). Formulations were subjected to ATF and temperature was maintained to 37 °C on a thermostatically controlled water bath. The formulations at physiological condition showed Pseudo plastic behaviour as solutions transformed into gels with high viscosity, as shown in Figure 7 and 8.

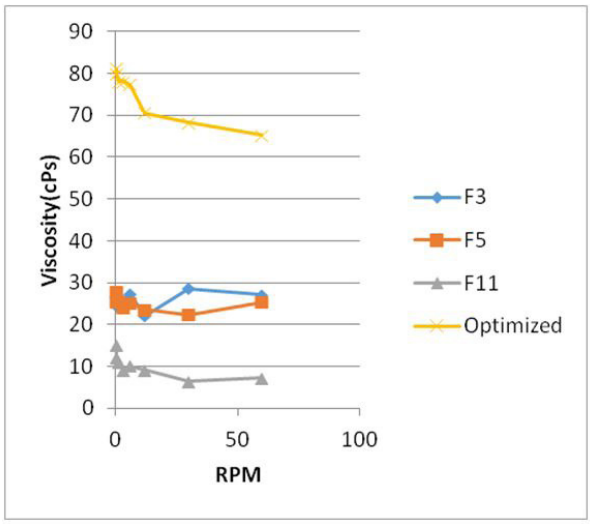

Figure 7: Viscosity of F3, F5, F11 and optimized formulation

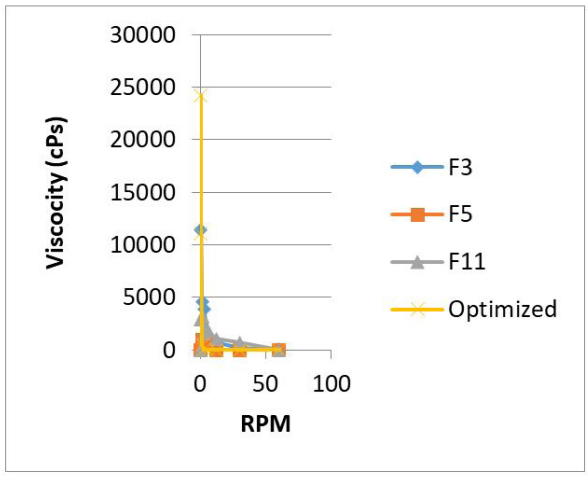

Figure 8: Viscosity of formulation F3, F5, F11 and optimized formulation in ATF

In vitro Release Study

The in vitro release studies of formulations were carried out with modified dissolution apparatus using cellophane membrane for a period of 8 hours. The in vitro release behavior was carried out with using artificial tear fluid (ATF) as a dissolution medium. The drug release study was observed at every 1 hour interval. The cumulative percentage release of drug at different time intervals is shown below (Figure 9 and 10).

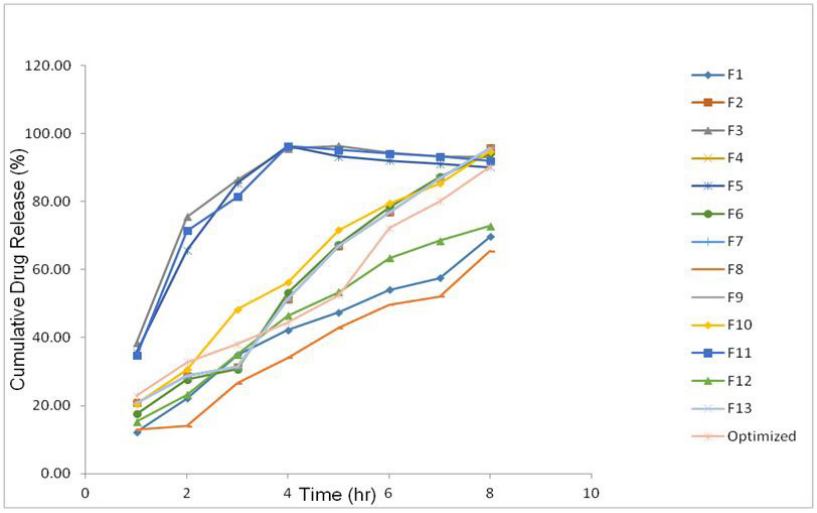

Figure 9: In-vitro drug release profile of formulations

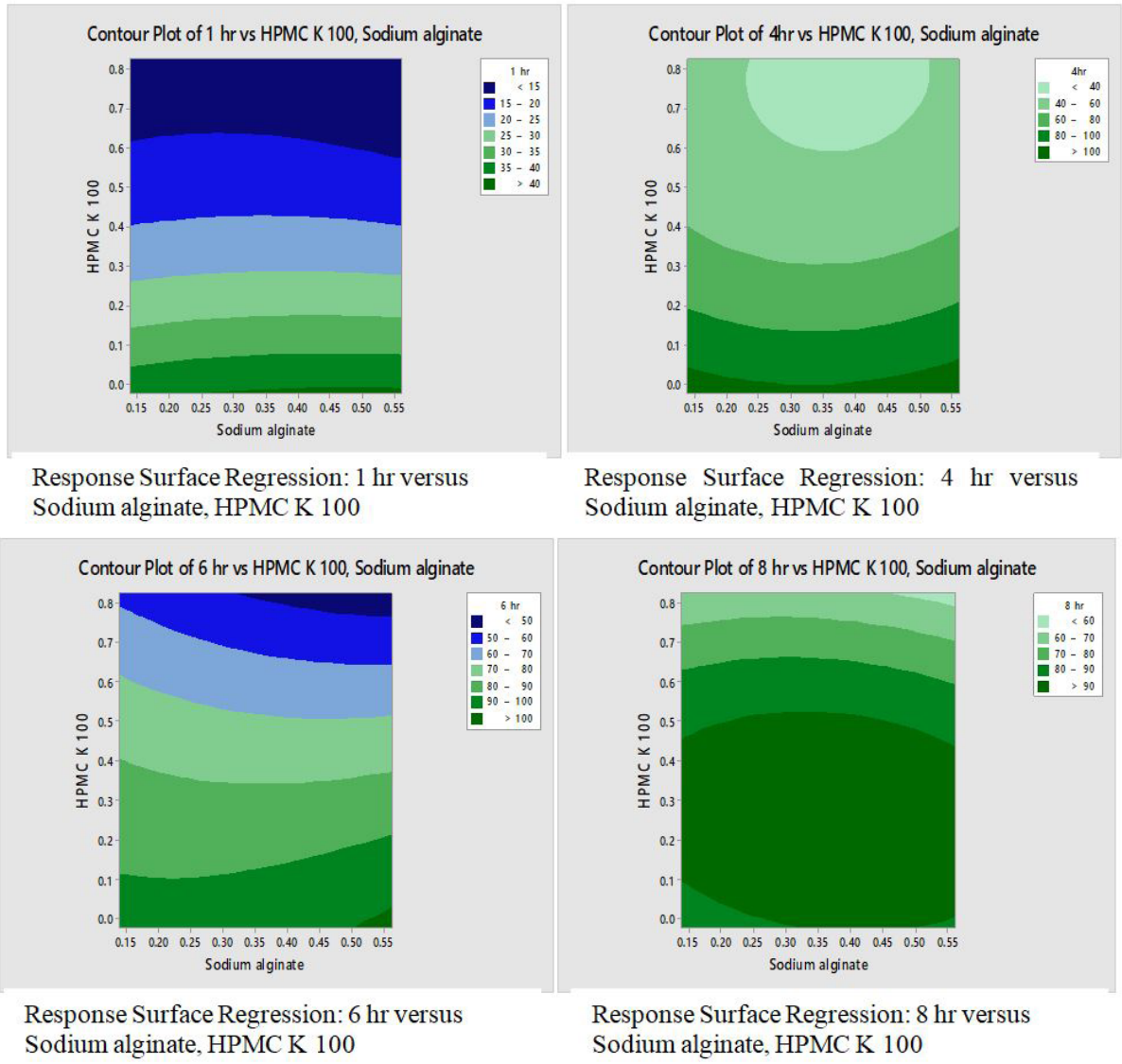

Figure 10: Contour Plot of release profile in 1st, 4th, 6th and 8th hour

Analysis of Response Surface Design

The results of in vitro drug release were analyzed at 1st, 4th, 6th and 8th hour. Equation derived from the regression coefficients at different time points are shown below:

Estimated regression coefficient at 1st hour:

$Y = 35.47+17.0X_1-55.05X_2-17.6X_1*X_1+32.68X_2*X_2-11.5X_1*X_2$

Estimated regression coefficient at 4th hour:

$Y = 17.7+179X_1+22.2X_2+23X_1*X_1+62.0X_2*X_2-360X_1*X_2$

Estimated regression coefficient at 6th hour:

$Y = -1.2+266\ X_1+152.0X_2-105X_1*X_1-64.6X_2*X_2-376X_1*X_2$

Estimated regression coefficient at 8th hour:

$Y = -15.1+346X_1+221.8X_2-242X_1*X_1-145.2X_2*X_2-331X_1*X_2$

Here, X_1 represents Sodium alginate and X_2 represents HPMC K 100

Negative coefficients of Sodium alginate suggests that it has significant effect in retarding the drug release whereas positive coefficients of HPMC K 100 confirms that HPMC K 100 alone does not have any significant effect in retarding the drug release.

The interaction between Sodium alginate and HPMC K 100 has shown negative coefficient in all time periods which suggests significant synergistic influence in retarding the drug release. The interaction between Sodium alginate with sodium alginate and HPMC K 100 with HPMC K 100 has shown synergistic effect in initial time periods.

Kinetic Study

The drug release kinetics of all thirteen formulated batches and optimized batch are given in Table. The best fitted model was selected upon coefficient of regression R^2. The coefficient of regression R^2 for zero order was obtained within a range of 0.7289 to 0.9796 when plotted cumulative percentage drug release against time up to 8 hours for 13 batches with 0.9752 for optimized batch. The R^2 for first order release kinetics was within 0.8332 to 0.979 when log cumulative percent drug remaining was plotted against time for all formulated batches with 0.8782 for optimized batch. R2 for Higuchi model was within 0.8339 to 0.9879 when cumulative percentage drug release was plotted against square root of time with 0.9291 for optimized batch. Similarly, Krosmeyer–Peppas equation provided correlation coefficient R2 value within range of 0.8857 to 0.988 with 0.994 for optimized batch. The release exponent "n" provided by power low were in the range of 1.1087 to 1.6485 with 1.3137 for optimized batch.

The formulations formed opaque matrix immediately upon addition to the dissolution medium, due to presence of ions in artificial tear fluid. Hence the release of drug from this matrix was possibly by diffusion and /or erosion of the matrix. The drug release pattern obtained for the gelled samples is characteristic of hydrophilic matrices. The initial fast release of Ciprofloxacin hydrochloride may be due to the fact that the in situ gels are formulated in water and hence the polymers (HPMC and Sodium Alginate) are completely hydrated. Hydrated Sodium alginate when come in contact with ATF leads to gelation. In this prehydrated matrix, water penetration is no longer limit for drug release leading to an apparent diffusion controlled release.

The regression coefficient (R^2) of zero order, first order, Higuchi matrix and Krosmeyer-Peppas and 'n' value of Krosmeyer-Peppas are tabulated in Table 8 below

FN	Zero Order	First Order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	N
F1	0.9796	0.9721	0.9879	0.988	1.1087
F2	0.9796	0.8799	0.9498	0.9426	1.2483
F3	0.7289	0.8332	0.8339	0.8896	1.5807
F4	0.8578	0.9536	0.9372	0.9477	1.4695
F5	0.7389	0.8488	0.8394	0.886	1.4968
F6	0.7421	0.8561	0.8432	0.8865	1.4997
F7	0.7769	0.8759	0.8691	0.9137	1.5944
F8	0.853	0.979	0.9154	0.9146	1.4186
F9	0.7374	0.846	0.8378	0.8857	1.5014
F10	0.8629	0.9623	0.9406	0.9192	1.373
F11	0.7354	0.8423	0.8377	0.8866	1.5069
F12	0.8966	0.9853	0.9625	0.9496	1.4243
F13	0.7698	0.9054	0.8716	0.9137	1.6485
Optimized	0.9752	0.8782	0.9291	0.949	1.3137

Table 8: Drug Release Kinetics of formulated batches and optimized batch

Ciprofloxacin hydrochloride release from the in situ gel of the optimized formulation follows the Higuchi square root law and Krosmeyer-Peppas law with $R^2 > 0.83$.

Higuchi matrix model suggests that the drug release occurs by diffusion mechanism. The polymers can absorb a significant amount of water to form an elastic gel and, at the same time, release the dissolved entrapped drug by diffusion through swollen regions of the gel. Similarly, the release index (n) of the optimized formulations was 1.3137, which indicated that the formulation showed drug release by Non-Fickian diffusion mechanism.

Optimization of the Formulation

As per FIP guidelines for a formulation to be sustained or controlled release, dissolution specification should consist at least three points where 1st or 2nd hour should show the release of 20% to 30%, second specification should show the release of 50% and final specification point should show the release of not less than 80%. Similarly, based on various other research articles Prachi *et al.*, Talari Sivannarayana *et al.*, and Gupta *et al.*, the criteria for four time points are defined as in Table 9 below

Time points	Lowest	Target	Highest
1st hour	20	28	30
4th hour	60	65	70
6th hour	70	75	80
8th hour	Maximum Release		

Table 9: Dissolution criteria for optimization of formulation

For all the four responses, the optimized plot was obtained using response optimizer in minitab software as shown in Figure 11:

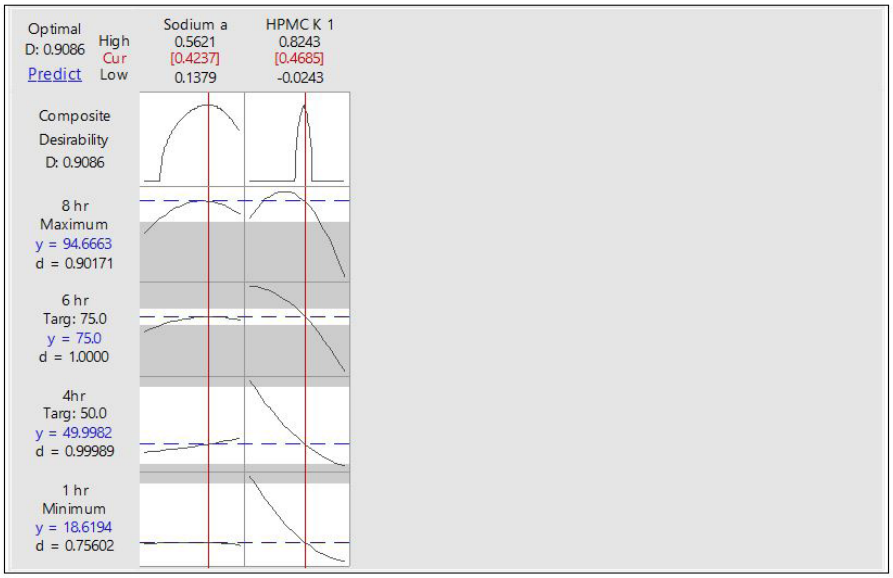

Figure 11: Optimization plot of in-situ gelling system

Comparison of Optimized Formulation with Marketed Formulation

The drug release of optimized formulation was compared with the conventional marketed eye drop Ciflox purchased from retail pharmacy. Same procedure as mentioned above was followed for the release study. The cumulative percentage release of optimized formulation and marketed formulation versus time were plotted as shown in Figure 12:

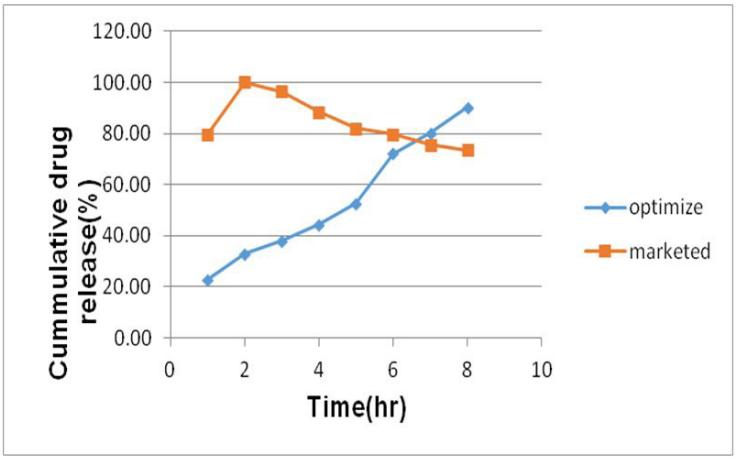

Figure 12: Comparison of Optimized formulation with marketed conventional eye drop

The conventional eye drop has shown complete drug release within 3 hours whereas optimized formulation sustained the drug release up to 8 hours. The rapid in vitro release of marketed formulation may be due to lack of any release retarding agents as in gelling system.

Sterility Test

Sterilization by autoclaving had no effect on the physical and chemical properties of the formulation. There was no appearance of any bacterial colonies in the plates when the plates were incubated for not less than 14 days at 37 °C. Hence, there was no evidence of microbial growth. The optimized formulation therefore passed the test for sterility and sterility was achieved by this technique without affecting the nature of formulation (Figure 13,14 and 15).

Figure 13: Negative Control

Figure 14: Optimized Formulation

Figure 15: Positive Control (Presence of bacterial growth)

Conclusion

The ion-sensitive ophthalmic in situ gel of Ciprofloxacin Hydrochloride was successfully formulated by using Sodium alginate as gelling agent and HPMC as viscosity enhancer and release retardant. The formed gels were stable for the period of 8 hours and provided delayed release. Response surface design, regression analysis, contour plots, and desirability function obtained from response optimizer have been proven to be a useful approach for the optimization of formulations. The optimized formulation has shown sustained drug release over the period of 8 hours. So, this formulation can be used as an alternate to conventional eye drops to improve the bioavailability through its longer precorneal residence time and ability to sustain drug release.

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