FORMULATION AND EVALUATION OF A BIODEGRADABLE *IN SITU* GEL OF AN ANTIBACTERIAL DRUG FOR CONTROLLED OCULAR DRUG DELIVERY

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Abstract*: The study aimed to formulate and evaluate a biodegradable *in situ* gel of an antibacterial drug for controlled ocular drug delivery. Ciprofloxacin hydrochloride is a broad-spectrum antibiotic used in the treatment of corneal ulceration and ocular infections. Even though drug instillation into the eye by using conventional methods is easy these have certain disadvantages like rapid dilution and washout (due to the presence of tear) which leads to poor retention time of drug in the eye. To overcome these disadvantages an *in situ* gelling system can be designed. Here we can overcome these disadvantages of conventional drug administration and also, we can retain a higher drug concentration in the eye for a longer period of time. The *in situ* gel formulated will be in a liquid state once instilled into the eye it is transformed into gel. This transition is due to the presence of thermosensitive polymers added in the formulation. Here two different variants of thermosensitive polymers poloxamer namely, poloxamer 188 and poloxamer 407 have been used in the ratio 15:23 to obtain a sustained drug release from the *in situ* formed in the eye. Further HPMC K4M and chitosan in ratio 1:1 was added to the formulation to control the burst release and to obtain a controlled ocular delivery. The final formulation selected with optimum controlled release was subjected to evaluation such as clarity, pH, thermosensitive evaluations, viscosity, drug content, drug entrapment efficiency, and *in-vitro* drug release study. From the evaluation of results, it could be concluded that the final formulation FRP-II was able to pass all the evaluation tests and could sustain the drug release upto a period of 7hr. The results of kinetic study revealed that the formulation has non-fickian diffusion-controlled drug release. From the results of accelerated stability studies, the batch was found to be stable. So, it can be concluded that a combination of poloxamer 188 and 407 along with rate controlling polymers HPMC K4M and chitosan can be successfully used to formulate *in situ* gel system of Ciprofloxacin hydrochloride for controlled ocular drug delivery.

***Keywords*: *in situ* gel, poloxamer 407, poloxamer 188, sol-gel transition**

Introduction

The most prevalent dosage form for the treatment of eye infections are eye drops. 90% of the marketed formulation of eyedrops are safe, easy to administer and are patient compliant. However, there are numerous physiological barriers in the structure of eye that limits ocular drug bioavailability. As a result, only 5% of commercially available drugs can penetrate intraocular tissue. The main objective of formulation was to permeate the drug through the protective layer without damaging ocular tissues while achieving the minimal effective drug concentration of the drug for a prolonged time, at the site of action. Conventional eye drops are associated with disadvantages like quick precorneal clearance, small absorptive surface area which leads to loss of drug, and poor bioavailability.[1]

To overcome the disadvantages and as a further advancement to eye drops, topical gels, ointments, hydrogel, and insert were introduced into the market. Even though these dosage forms succeeded in attaining better bioavailability due to increased contact time they were associated with disadvantages like blurring of vision, difficulty in administration, and lesser patient compliance.

Above mentioned disadvantages could be overcome by formulating the drug as an *in situ* gel formulation. *In situ* gelling systems use polymers that exhibit a sol-gel phase transition in response to a stimuli such as temperature, pH, chemical change etc. Therefore, the drug which is in the solution form will be transformed into gel upon instillation into the eye. Hence by formulating an *in situ* gel for ophthalmic drug delivery, we will be able to combine the advantages of ease of administration and retention of dosage form which could sustain the drug release for a longer period of time. [2,3,4]

The present work describes the formulation and evaluation of an *in situ* gel of an antibacterial drug, Ciprofloxacin hydrochloride using biodegradable polymers for controlled ocular drug delivery. In which *in situ* phase transition occurs on the surface of the cornea by the temperature triggering mechanism.

Ciprofloxacin hydrochloride is an antibacterial drug used in the treatment of corneal ulceration and conjunctivitis caused by susceptible strains of bacteria including, *Pseudomonas aeruginosa, Serratia marceescens, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumonia, Strepto-coccus Haemophilus influenzae*.[5,6]

The other excipients used in the formulation were polymer (Poloxamer) copolymers (HPMC K4M and Chitosan) and excipients such as NaOH, Benzalkonium chloride etc.

MATERIALS AND METHODS

2.1 Materials

Ciprofloxacin hydrochloride, Poloxamer 407, Poloaxmer 188 were obtained from Yarrow medical, Maharastra. HPMC K4M and Chitosan were supplied from Balaji. All other chemicals and solvents were of analytical grade were used.

2.2 Analytical Methods[8]

2.2.1 Determination of λ_{max}

Ciprofloxacin hydrochloride was dissolved in simulated tear fluid pH 7.4 (STF) to produce a 10 µg/ml solution. λ_{max} of this solution was measured by using UV spectrophotometer in the range of 200-400nm.

2.2.2 Standard curve of Ciprofloxacin hydrochloride

A stock solution of Ciprofloxacin hydrochloride 100 µg/ml was prepared using STF. This solution was further diluted to obtain 2, 4, 6, 8, and 10 µg/ml solution, and the absorbance was measured by using UV spectroscopy at 272nm.

2.3 Preformulation studies

2.3.1 Determination of melting point[9,10]

The melting point of the drug was determined by using Thiel's apparatus by the open capillary method.

2.3.2 Determination of Solubility[11,12]

The solubility of the drug was determined by the supersaturation method. 2ml of the solvent was taken in test tube and an excess amount of drug was added, shaken for 24hrs and filtered. The supernatant was diluted and absorbance was measured using UV spectroscopy at 272nm.

2.3.3 Drug excipient compatibility studies[12]

The drug excipient compatibility studies were carried out for the drug and all the polymer in the ratio 1:1, for physical mixture, and also for the final formulation to rule out any incompatibility between the drug and excipients used in the preparation. The compatibility study was done by FT-IR spectroscopy by using the KBr pellet technique.

2.4 Formulation of *in situ* gel[12-15]

The gel was formulated by the cold method suggested by Schmolka. The required vehicle was chilled to 4°C. The polymer is mixed with vehicle by using a homogenizer to obtain a uniform distribution and kept overnight for complete solvation and to get a clear solution. The Ciprofloxacin hydrochloride and benzalkonium chloride were dissolved in a small quantity of water and mixed with the polymeric solution at 2000 rpm for 30 min. The pH was adjusted to 6.8-7.4 and solution was stored at 4°C. Different polymers used in the formulation are selected by the trial-and-error method.

Six combinations of poloxamer polymers, poloxamer 188 and 407 which are thermosensitive were selected based on their gelling temperature which ranged from 32-35°C (the physiological temperature of the eye).

2.5 Evaluation studies for the developed formulations

2.5.1 Physical appearance[16-18]

The prepared solutions were visually examined for any foreign body or lumps against a black and white background.

2.5.2 pH[17-19]

The pH of the prepared formulations were evaluated by using a digital pH meter.

2.5.3 Thermosensitive evaluations[19-22]

2.5.3.1 Gelation temperature

Gelation temperature refers to the temperature at which a drug solution becomes a gel. It was carried out by the tube inversion method. 2ml of sample transferred into a test tube and placed in a water bath with a thermostat to maintain the specific temperature. The gelation temperature was noted when the solution has formed a thick gel when it was tilted at 90°.

2.5.3.2 Gel melting temperature

For this study, the temperature of the solution was further increased and the temperature at which the gel melted was noted. Gel melting temperature is required to confirm that the formed gel will not melt at the body temperature.

2.5.3.3 Gel duration[21-22]

Gel duration study was conducted to determine the time duration upto which the developed gel remains intact in the eye, which is a requisite for sustaining the drug release. 2ml of the test solution was taken in a test tube and placed in a water bath maintained at 35±0.5°C. The time it takes for the gel to convert into solution was noted.

2.5.3.4 Gelation time[23-24]

Gelation time refers to the time taken by test solution to transform into a gel when no flow criteria of the meniscus is observed. It was performed by the tube inversion method. 2ml of test solution was taken in a test tube and maintained at its gelation temperature. The time taken for the test solution to form a stiff gel was noted.

Table No1: Formulation of Ciprofloxacin Hydrochloride loaded *in situ* gel

Formulation batch	Drug (% w/v)	Poloxamer 188 (% w/v)	Poloxamer 407 (% w/v)	Benzalkonium Chloride (ml)	Distilled water (ml)
F20	0.3	10	22	0.01	100
F21	0.3	15	22	0.01	100
F22	0.3	5	23	0.01	100
F23	0.3	10	23	0.01	100
F24	0.3	15	23	0.01	100
F27	0.3	15	24	0.01	100

2.5.4 Viscosity[18,17]

Viscosity is used to determine the rheological behavior of the prepared formulation both in solution and gel form. The viscosity was measured by using Brookfield viscometer LVDV prime I at temperatures 27±2 ºC and 35±0.5ºC. Spindle No 62 was used. It was selected based on the trial-and-error method.

2.5.5 Drug content[8,25]

1ml of prepared drug solution was taken and dissolved in STF and makeup to 100ml solution. The drug concentration was determined by using UV spectrophotometer. Drug content was determined by the equation

$$\text{Drug content} = \frac{Concentration\ from\ graph}{1000} \times Dilution\ factor$$

2.5.6 Percentage drug entrapment efficiency[26]

Amount of gel equivalent to 0.3mg was taken and dissolved in small quantity of distilled water then volume was adjusted to 100ml with STF. From this solution 5ml was pipetted out and absorbance was measured using UV spectrophotometer at 272nm. The percentage entrapment efficiency can be calculated by the following equation.

$$\text{Drug entrapment efficiency (\%)} = \frac{Amount\ of\ drug\ in\ solution}{Theoretical\ drug\ content} \times 100$$

2.5.7 *In-vitro* drug release[27-28]

In-vitro drug release study was performed by using a modified Franz diffusion cell. Cellophane membrane was immersed in STF for 24hrs. The details of the study are as follows:

Dissolution medium: STF

Dissolution medium volume :100ml

Apparatus used: Modified Franz diffusion cell

RPM: 100

Temperature: 35±0.5°C

2ml sample was withdrawn at time intervals, 30, 60, 120, 180, 240, 300, 360, 420, 480 min and was replaced with 2ml fresh medium. Absorbance of these samples were measured at 272nm by UV- visible spectroscopy.

2.5.8 Formulation of *in situ* gel by using rate controlling polymer.[29-32]

To control the burst release, a combination of rate controlling polymers i.e., chitosan (1%w/v) and HPMCK4M (0.5-1.5%w/v) were used. All the evaluation studies were performed once again for the final optimized batch, to study the effect of addition of rate controlling polymers to the formulation.

Table No.2 Formulation of *in situ* gel containing rate controlling polymers

Ingredients	FRPI	FRPII	FRPIII
Drug %w/v	0.3	0.3	0.3
Polxamer 188 %w/v	15	15	15
Poloxamer 407 %w/v	23	23	23
Benzalkonium chloride ml	0.01	0.01	0.01
Chitosan %w/v	1	1	1
HPMC K4M %w/v	0.5	1	1.5
Distilled water ml	100	100	100

2.5.9 Drug release kinetics[33-35]

To determine the release mechanism from formulation, the regression coefficients of various kinetic models were performed, including zero-order, first-order, Higuchi model, Hixson Crowell, and Korsmeyer-Peppas.

2.5.10 Accelerated stability studies[37-38]

Accelerated stability studies was performed for the final batch to ensure the product efficacy, safety, and quality of active drug substances in dosage form during the shelf life. The optimized formulation was subjected to stability studies as per ICH guidelines.

3.0 Results and Discussion

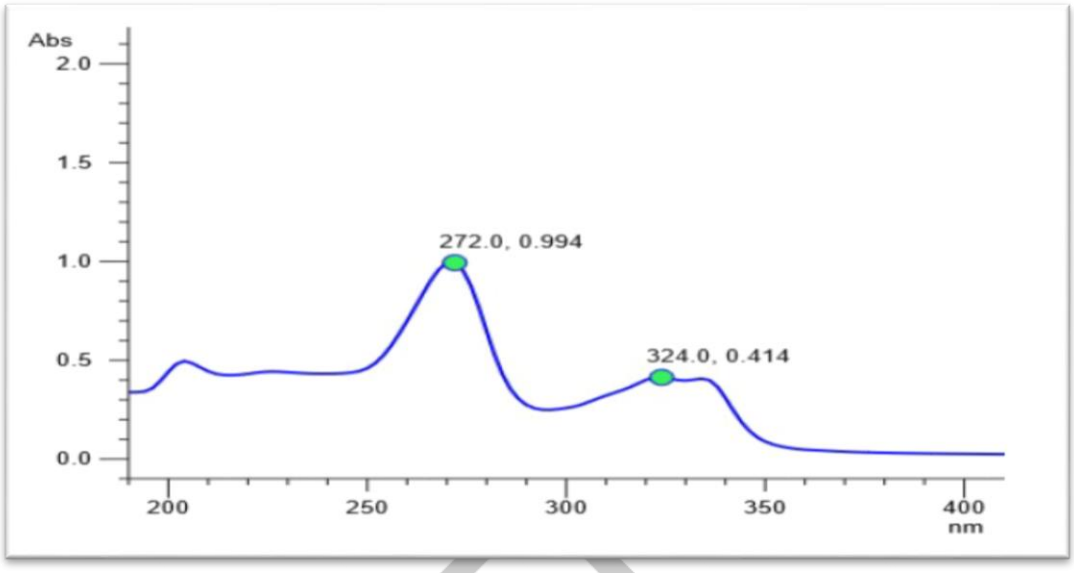

Fig No.1 λ_{max} of Ciprofloxacin hydrochloride

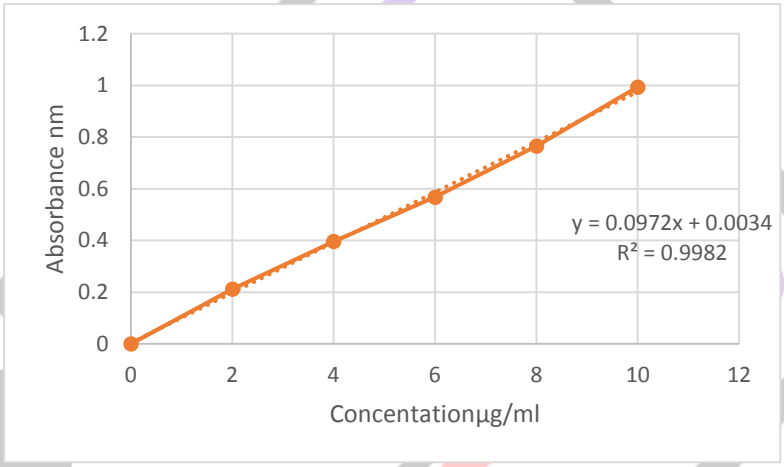

Fig No.2: Calibration curve of Ciprofloxacin hydrochloride

Preformulation studies

Melting point of Ciprofloxacin hydrochloride was found to be 255ºC which complies with the official standards.

Solubility: The drug showed a maximum solubility of 37.5 mg/ml in water and 1.75 mg/ml in STF.

FT-IR

Results for FT-IR study showed that there was no interaction between the Ciprofloxacin hydrochloride and polymers. As per the results of FT-IR studies, there was no new peaks or disappearance of existing peak suggested that Ciprofloxacin hydrochloride was compatible with all the excipients used and also with the method of preparation.

Table No.3: Results for the thermosensitive evaluations

Formulation batch	Clarity	pH	Thermosensitive evaluations			
			GT*(°C)	GM***(°C)	Gel Time (Sec)	Gel Duration(hr)
F20	Clear	6.93±0.02	34.4±0.15	45.5±0.23	30.5±0.5	2.5±0.25
F21	Clear	7.01±0.01	33.5±0.23	56.5±0.15	25.6±0.3	4±0.45
F22	Clear	6.87±0.03	32.5±0.42	50.5±0.32	17.7±0.2	5±0.32
F23	Clear	6.88±0.02	33.6±0.54	65.5±0.42	21.0±0.1	7±0.56
F24	Clear	7.04±0.01	33.2±0.33	70.5±0.14	16.0±0.4	10±0.89
F27	Clear	7.12±0.01	34.4±0.15	65.5±0.52	12.5±0.24	12±0.75

GT*- Gelation temperature GM**- Gel melting temperature

Evaluation of Ciprofloxacin hydrochloride loaded *in situ* gel

Physical appearance: The prepared solutions were clear and free from foreign particles

pH: All the developed formulations showed a pH range of 6.8-7.2 which was found to be compatible with pH of eye.

Thermosensitive evaluations:

For the 6 batches, thermosensitive evaluations were carried out and the results are given in Table No.3. From the data, the gelation temperature was varied from 32.5- 34.4°C. The gel melting temperature was ranged from 45.5-70.5°C. All 6 formulations showed a gelation time which was found to be satisfactory. Gel duration of more than 5 hrs was exhibited by the batches F23, F24 and F27 which was selected for the further studies.

Viscosity:

Viscosity showed a linear relationship with the polymer used in the preparation. The viscosity of the formulation at 35±0.5°C was found to be high when compared with 27±2°C. The increase in the viscosity at physiological temperature suggests that the prepared formulation exhibited a sol to gel transformation. Sol to gel transformation possesses pseudoplastic rheologic characteristics.

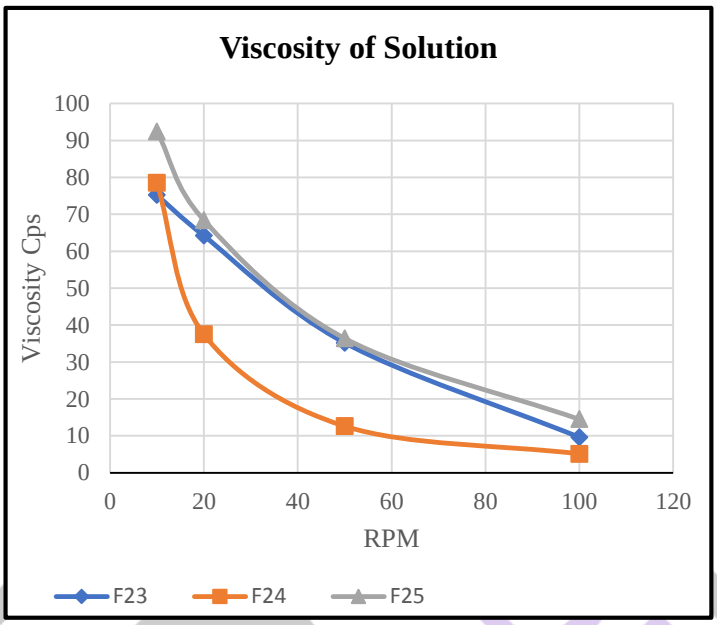

Fig No.3: Viscosity of solution

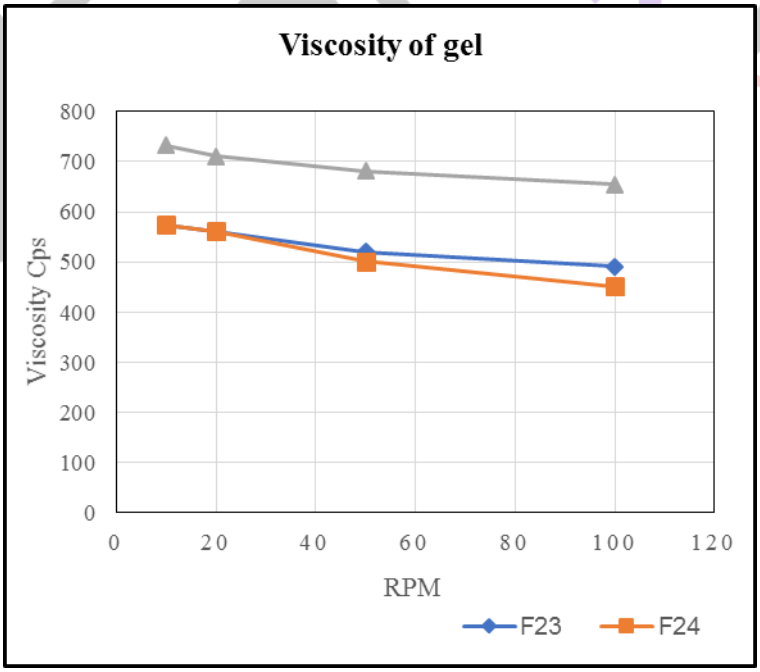

Fig No.4: Viscosity of gel

Drug entrapment efficiency:

The percentage of drug entrapped varied from 72.6±0.50 – 80.2±0.36% which was satisfactory.

Drug content:

The drug content of the developed formulation ranged from 92.4±0.28 to 98.5±0.64% which was satisfactory.

***In-vitro* drug release study**

Results for the *in- vitro* drug release study were tabulated in Table No.4 and Fig No.5.

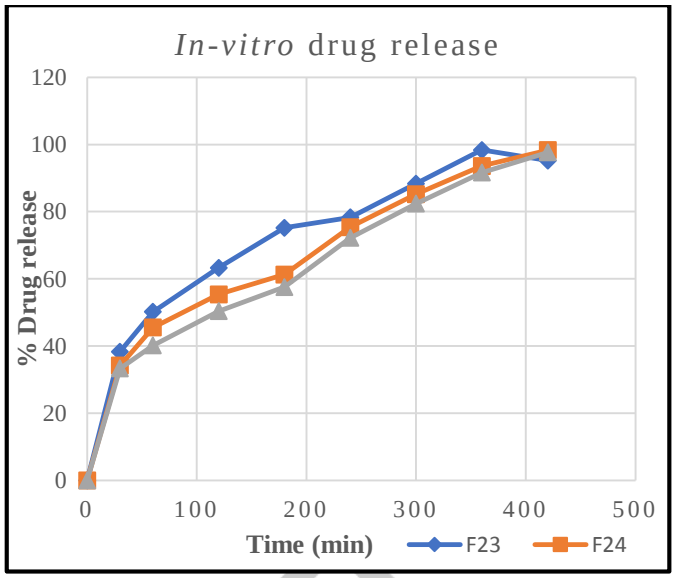

Fig No.5: In vitro drug release study

F23 showed a cumulative drug release of 98.34% at 360 min (6hr). whereas F24 and F27 showed a cumulative drug release of 98.33 and 97.66% respectively till 420 min (7hr). F24 was selected over F27 as it had lesser concentration of polymers and extended the drug release to the same extend as F27. But F24 batch was further studied to optimize the burst control by incorporating rate-controlling polymers.

Table No. 4: *In vitro* drug release study

Time (min)	% Cumulative drug released*		
	Formulation batch		
	F23	F24	F27
0	0	0	0
30	38.33±0.12	34.23±0.19	33.33±0.15
60	50.2±0.45	45.53±0.23	40.23±0.53
120	63.33±0.23	55.42±0.52	50.4±0.35
180	75.2±0.19	61.32±0.45	57.6±0.26
240	78.32±0.69	75.42±0.25	72.23±0.69
300	88.34±0.43	85.23±0.62	82.4±0.48
360	98.34±0.51	93.62±0.48	91.66±0.24
420	95.21±0.33	98.33±0.52	97.66±0.55
480	92.75±0.52	97.66±0.33	95.2±0.19

3 different trials were done as per the formula given in Table No.2. The combination of chitosan and HPMC K4M were selected to control the burst release from the gel. The concentration of chitosan was fixed to a 1%w/v and the concentration of HPMC K4M varied from the concentrations 0.5-1.5%w/v.

In-vitro drug release was performed once again to study the effect of rate-controlling polymers. The results were tabulated in Table No.5 and Fig No.6

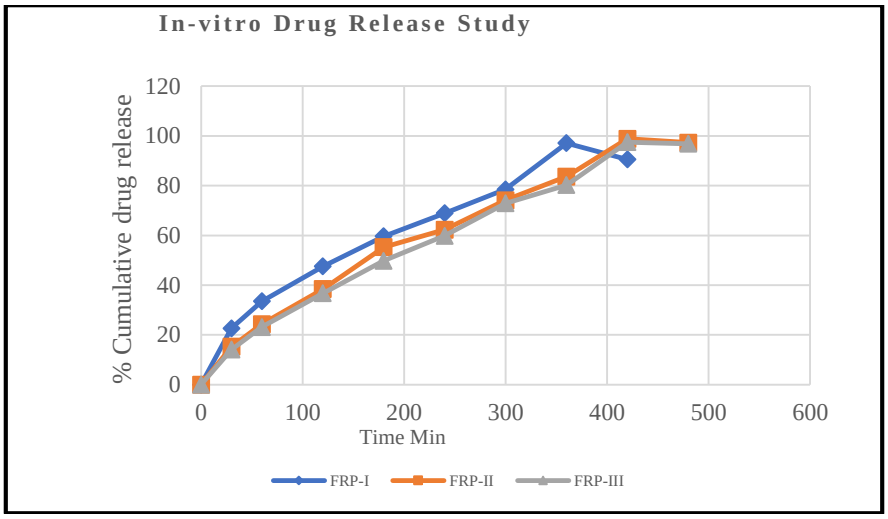

Fig No.6: *In vitro* drug release study after incorporation of rate-controlling polymers.

Table No.5: *In-vitro* drug release study with rate controlling polymers

Time (Min)	% Cumulative drug released		
	FRP-I	FRP-II	FRP-III
0	0	0	0
30	22.63±0.15	15.24±014	14.12±0.32
60	33.54±0.23	24.32±0.53	23.14±0.25
120	47.54±0.55	38.43±0.25	36.73±0.41
180	59.64±0.42	55.26±0.30	49.82±0.23
240	68.89±0.12	62.26±0.41	59.84±0.16
300	78.53±0.25	74.14±0.15	72.92±0.43
360	97.14±0.35	83.65±0.24	80.24±0.35
420	95.52±0.54	98.84±0.45	97.52±0.52
480	88.73±0.24	97.31±0.35	96.83±0.46

FRP-I showed a cumulative drug release of 97.14% at 360min. FRP-II and FRP-III the cumulative percentage drug release was found to be 98.84 and 97.52% respectively at 420 min. These batches didn't exhibit any significant increase in drug release when the concentration of the rate-controlling polymer was increased.
FRP-II which has a lesser ratio of polymer was selected and finalized.
As per the results of the evaluation for the FRP-II batch, there was no significant difference in the evaluation parameters due to the addition of rate-controlling polymers.

Drug release kinetics

The release data obtained from the *in situ* gel were fitted into various models. The regression value (R^2) was compared for zero-order and first-order which was found to be 0.981 and 0.75 respectively. The R^2 values of Higuchi and Hixson Crowell were found to be 0.9744 and 0.9047 respectively. In the Korsmeyer peppas model the release exponent (n) value was found to be 0.7508. Thus, we can conclude that the drug release mechanism of zero-order kinetics, non-fickian diffusion-controlled pattern.

Table No.6: Drug release kinetics data

Formulation code	Zero-order	First-order	Higuchi model	Hixson Crowell model	Korsmeyer-Peppas model	
	R^2	R^2	R^2	R^2	R^2	N
FRP-II	0.9813	0.75	0.9744	0.9047	0.9983	0.7508

Accelerated stability studies

The FRP-II formulation was subjected to the accelerated stability studies. The physical appearance, drug content, pH, and *in-vitro* drug release study were carried out and results showed that there was no significant change from the previously obtained data.

Conclusion

We can conclude that Ciprofloxacin hydrochloride can be successfully formulated as an *in situ* gel for the controlled ocular drug delivery. The formulation FRP-II containing poloxamer 188 and 407 in the ratio 15:23 %w/v along with rate controlling polymers chitosan and HPMC K4M in the ratio 1:1 was able to deliver a controlled release of Ciprofloxacin hydrochloride from the *in situ* gel formed. The kinetic study proved that the drug release follows zero-order kinetics with non-fickian diffusion controlled pattern of drug release. The drug formulation can be subjected to further clinical studies to prove the theoretical anticipation of improved bioavailability with a controlled release of the drug.

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