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DEVELOPMENT AND EVALUATION OF PH TRIGGERED *IN-SITU* OPHTHALMIC GEL FORMULATION OF OFLOXACIN

P. B. Patel[1], D. H. Shastri[*1], P. K. Shelat[1], A. K. Shukla[1]

1. Department of Pharmaceutics, K. B. Institute of Pharmaceutical Education & Research, Gandhinagar, Guj, India.

ABSTRACT

Formulation and characterization of an ophthalmic delivery system of an antibacterial agent, ofloxacin, based on the concept of pH triggered *in situ* gelation. Polyacrylic acid (Carbopol 974P) was used as the gelling agent in combination with Noveon® AA-1 USP Polycarbophil as a viscosity enhancer. Formulation optimization was carried out using a 3^2 full factorial design. The effect of independent variables was evaluated using dependent variables such as gel strength, bioadhesive force, viscosity and *in vitro* drug release of the formulation. The multiple regression analysis of the results led to equations that adequately describe the influence of the independent variables on the selected responses. Polynomial regression equations and surface plots were used to relate the dependent and independent variables. Furthermore, the desirability function was employed in order to determine the best batch which was then evaluated for *in vivo* antimicrobial efficacy study, effect of sterilization, ocular irritation study and accelerated stability study. It was found that the optimum values of the responses for pH triggered *in situ* ophthalmic gel formulation could be obtained at medium levels of Carbopol 974P and Noveon® AA-1 USP Polycarbophil (0.5/0.5%w/w respectively). The formulation retained antimicrobial efficacy, showed insignificant effect over sterilization and found non irritant to the corneal surface confirmed by microscopy of the corneal mucosal membrane compared with reference marketed formulation. The optimized formulation was found to be stable, therapeutically efficacious and providing sustained release of the drug over an 8 h period even after accelerated stability study over three months. The developed system is thus a viable alternative to conventional ophthalmic formulations.

Key words: In situ gelation; Factorial design; Desirability function; Carbopol 974P;Noveon® AA-1 USP Polycarbophil; Ofloxacin

*Corresponding Author Email: divyeshshastri@gmail.com

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INTRODUCTION

Ophthalmic drug delivery is extremely interesting and highly challenging endeavors [1, 2]. The landscape of ophthalmic drug delivery is highly competitive and rapidly evolving. New classes of pharmaceuticals and biological are fuelling the demand for novel drug delivery systems. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. It is a common knowledge that, the ocular bioavailability of drugs applied topically as eye drops is very poor. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of eye and by other concomitant factors.

Nowadays, *in situ* gel forming systems are of great importance, having the combined advantage of being patient convenient with favorable residence time for enhancing ocular bioavailability and for reducing systemic side effects. The sol-gel transition can be induced by a shift in the pH (Carbomer)[3] temperature (Poloxamer) or by the presence of deacetylated gellan gum cations (Gelrite)[4].

The objective of the present work was to develop a pH triggered *in situ* gelling ophthalmic delivery system of Ofloxacin, a second generation Fuoroquinolone used in external infections of the eye such as acute and sub acute conjunctivitis, bacterial keratitis and keratoconjunctivitis. The topical ophthalmic dose of Ofloxacin is 1-2 drops of a 0.3%w/v solution in the affected eye(s) every 4 hrs in case of severe infection. A combination of Carbopol 974P and Noveon® AA-1 USP Polycarbophil were investigated as vehicle for the formulation of eye drops of Ofloxacin which would gel when instilled into the eye and provide sustained release of the drug during treatment in ocular infections.

MATERIALS AND METHODS

Ofloxacin was obtained as a gift sample from Bombay Tablet, Carbopol 974P and Noveon® AA-1 USP Polycarbophil were gifted by Lubrizol advanced materials.

Carbopol 974 P solution and Noveon® AA-1 polycarbophil solution were prepared by gradually dispersing the required amount of polymer in 0.01% BKC solution (precold to <10°C) with continuous stirring for 1 h. The Noveon® AA-1 polycarbophil solution and partially dissolved Carbopol 974 P solution were stored in the refrigerator until the entire polymer was completely dissolved (approximately 24 h). The in situ gel-forming solutions were prepared by mixing weighed quantities of Carbopol 974 P solutions with the Noveon® AA-1 polycarbophil solutions

in glass vials. The pH of the samples was then adjusted to 6.0±0.1 by the addition of measured volumes of a 0.5 M sodium hydroxide solution. After the polymer solutions were mixed, 0.01% BKC solution was added to obtain a final polymer concentration.

Mannitol was added into the formulations as the osmotic agent. The vials were tightly capped, and the samples were allowed to equilibrate for 24 h in refrigerator prior to the experiments. For preparation of 0.3% w/v Ofloxacin in situ gel-forming system, the desired amounts of Ofloxacin were dispersed to the Carbopol 974 P/Noveon® AA-1 polycarbophil solutions with continuous stirring until thoroughly mixed. All the sample solutions were adjusted to pH 6.0±0.1 or 7.4±0.1 by 0.5 M sodium hydroxide solution and then stored in the refrigerator prior to the evaluation of their rheological properties. Sterilization of 0.01% BKC and Carbopol 974 P/Noveon® AA-1 polycarbophil solutions was done by autoclaving at 121°C for 20 min. The final optimized formulations were formulated in aseptic conditions under laminar flow.

Drug content uniformity study

Drug content of ofloxacin was determined by dissolving an accurately weighed quantity of formulation (1 g in 50 ml) in pH 6.0 citro-phosphate buffer. The solutions were then filtered through 0.45 µm membrane filter and analyzed for ofloxacin content by UV spectrophotometer at 288 nm.

***In vitro* gelling capacity study**

The gelling capacity was determined by placing a drop of the system in a petridish containing two ml of Simulated Tear Fluid (STF) freshly prepared (sodium chloride 0.67 g, sodium bicarbonate 0.2 g, calcium chloride dihydrate 0.008 g in a purified water q. s. to 100 mL) with pH 7.4 equilibrated at 37˚C. Visual assessment of the gel formation was carried out. Time required for the gelation and the time taken for the formed gel to dissolve was taken as the evaluation parameters.

Rheological study[8]

Rheological studies of the formulations were carried out using the Brookfield viscometer (Brookfield model R/S CPS, USA). The pH of the solutions was raised from 6.0 to 7.4 by neutralizing with 0.5M NaOH, and simultaneously, the temperature of the solution was increased from 25°C to 37 °C. The viscosity of the samples was recorded before and after gelling. Each experiment was performed in triplicate.

In vitro drug release study

The in vitro release of Ofloxacin from the formulation was studied using a modified USP XXIII dissolution testing apparatus (TDT-08L, Electrolab, India). Freshly prepared Simulated Tear Fluid (STF; pH 7.4, ionic strength of 0.188) was used as the dissolution medium. Simulated Tear Fluid (STF) was made using NaCl 0.67 g, $NaHCO_3$ 0.20 g, $CaCl_2$ $2H_2O$ 0.008 g, and distilled deionized water to 100 g [9, 10]. Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter) A one ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37±1˚C so that the membrane just touched the receptor medium surface. The shaft was rotated at 50 rpm. At hourly time intervals, 5 ml of solution was withdrawn from the cell and replaced with an equal volume of fresh dissolution medium to provide sink condition. The samples were diluted with the receptor medium and analyzed by UV spectrophotometer at 288 nm. Each experiment was performed in triplicate

Antimicrobial efficacy studies

This was determined by the agar diffusion test employing 'cup plate technique'. Sterile solutions of ofloxacin in citron phosphate buffer, pH 6.0 (standard solutions) and the developed formulation diluted suitably with citrophosphate buffer, pH 6.0 (test solutions) were poured into cups bored into sterile nutrient agar previously seeded with test organisms (P. *aeruginosa* and S. *aureus*). After allowing diffusion of the solutions for 2 h, the agar plates were incubated at 37˚C for 24 h. The Zone of Inhibition (ZOI) measured around each cup was compared with that of control. The entire operation except the incubation was carried out in a laminar air flow unit. Each solution was tested in triplicate. Both positive and negative controls were maintained through the study.

Adhesiveness study[11]

The adhesion study of controlled release ofloxacin formulations, after gelling, was measured using the apparatus described by Agrwal and Mishra et al. The apparatus consisted of two circular aluminum discs each of 3 cm diameter. One disc was allowed to hang on an iron stand with the help of an aluminum wire fastened with a hook provided on the back side of the disc. The other disc was connected to a pre weighed lightweight plastic bag using a hook attached on its back. The studied gel was placed between the two discs, which were then kept under constant pressure for 5 min (preload time) to initiate adhesion bond. After that, water was added to the plastic bag through an intravenous infusion set at a rate of 1 drop/min until the two adhered discs

became detached from each other. The water collected in the bag was weighed. The weights of collected water were converted into force required for detachment.

Effect of Sterilization study [12-14]

The selected formulations were filled in 50 ml capacity amber glass bottles, closed with grey butyl rubber closures and sealed with aluminum caps. The vials were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min. The formulations were evaluated for drug content, viscosity, clarity and pH before and after the terminal sterilization.

Accelerated Stability Study [13-14]

In situ ophthalmic gel of optimized batch was subjected to stability study. The formulations were stored at 50±1˚C for a period of 2 month. The formulation was finally evaluated for the drug content, clarity, viscosity, pH and gelling capacity.

Factorial Design for optimizing the in situ Ophthalmic Gel

To study all the possible combinations of all factors at all levels, a two factor, three level full factorial design was constructed and conducted in a fully randomized order (Table 1). The dependent variables measured were viscosity at pH 6.0 & 7.4, adhesiveness and percent drug release in simulated tear fluid (pH 7.4).

Table 1: Composition of 3^2 Factorial designs

Ingredients (%)	A1	A2	A3	A4	A5	A6	A7	A8	A9
Ofloxacin	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Carbopol 974P	0.4	0.5	0.6	0.4	0.5	0.6	0.4	0.5	0.6
Noveon AA-1 USP Polycarbophil	0.25	0.25	0.25	0.50	0.50	0.50	0.75	0.75	0.75
Citric acid	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407
Disodium hydrogen phosphate	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125	1.125
Mannitol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Distilled water q. s. to	100	100	100	100	100	100	100	100	100

Independent variables	Levels		
	Low (-1)	Medium (0)	High (+1)
Amount of Carbopol 974P (X_1) g	0.4	0.5	0.6
Amount of Noveon AA-1 USP Polycarbophil (X_2) g	0.25	0.50	0.75

Two independent factors, the concentration of Carbopol 974P (X_1) and the concentration of Noveon®AA-1 USP Polycarbophil (X_2) were set at three different levels. High and low levels of each factor were coded as +1 and -1, respectively and the mean value as zero. The range of a factor must be chosen in order to adequately measure its effect on the response variables. The

range of each factor was chosen from the preliminary studies. This design was selected as it provides sufficient degrees of freedom to resolve the main effects as well as the factor interactions. Stepwise regression analysis was used to find out the control factors that significantly affect response variables.

Equations relating independent variables and responses

The equations relating independent variables and responses were obtained by subjecting the results to statistical evaluation. Microsoft Excel version 2007 was used to perform multiple linear regressions to determine the control factors that significantly affect the responses.

Independent Variables: X_1: Carbopol 974P, **X_2:** Noveon® AA-1 USP Polycarbophil.

Responses: Viscosity at pH 6.0 & 7.4, bioadhesion study and cumulative percentage drug release.

Polynomial equation for 3^2 full factorial designs:

$$Yi = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

Where Y_i is the dependent variable; b_0 is the arithmetic mean response of all trials; and b_i is the estimated coefficient for factor X_i. The main effects, X_1 and X_2, represent the average value of changing factor one at a time; X_1X_2 represent the interaction terms and the polynomial terms (X_1^2 and X_2^2) are used to assess nonlinearity.

The significant factors in the equations were selected using a stepwise forward and backward elimination for the calculation of regression analysis. The terms of full model having non significant p value ($p>0.05$) have negligible contribution in obtaining dependent variables and thus are neglected.

Desirability Function

The application of the desirability function combines all the responses in one measurement and gives the possibility of predicting optimum levels for the independent variables. The desirability function was used for optimization of the formulation. During optimization of formulations, the responses have to be combined in order to produce a product of desired characteristics. The method was adopted to calculate the desirability of individual dependent variable and overall desirability by taking geometric mean. The batch having highest overall desirability (near to 1) value should be considered as an optimum batch.

The combination of the responses in one desirability function requires the calculation of the individual functions. As described earlier, a suitable *in situ* ophthalmic gel should have a

moderate adhesiveness, high viscosity at pH 7.4, low viscosity at pH 6.0 and high percent drug release after 8 hours.

Adhesiveness study

In this study, there were no specific requirements for adhesiveness of the optimum formulation. Therefore, the range was selected from the obtained responses. As moderate adhesiveness was desired for the *in situ* ophthalmic gel of Ofloxacin, the formulations having value within the range have desirability '1'. The formulations that have values outside this range have desirability '0'. It can be described by the following equations:

d_1 = '0' for $Y_i < Y_{min}$

d_1 = '1' for $Y_{min} < Y_i < Y_{max}$

d_1 = '0' for $Y_i > Y_{max}$

Where d_1 = the individual desirability of the adhesiveness.

Viscosity at pH 7.4 and Cumulative percentage drug release

The viscosity at pH 7.4 and percent drug release values were maximized in the optimization procedure, as suitable *in situ* ophthalmic gel should have high viscosity at pH 7.4 and high percent drug release. The desirability functions of these responses were calculated using the following equation:

$$d_2 \text{ or } d_3 = \frac{Y_i - Y_{min}}{Y_{target} - Y_{min}} \text{ for } Y_i < Y_{target}$$

d_2 or d_3 = 1 for $Y_i > Y_{target}$

Where d_2 = the individual desirability of viscosity at pH 7.4 and

d_3 = the individual desirability of percent drug release.

Viscosity at pH 6.0

The viscosity at pH 6.0 was minimized in the optimization procedure, as suitable *in situ* ophthalmic gel should have low viscosity at pH 6.0.The formulation should be easily droppable. The desirability functions of this response were calculated using the following equation:

$$d_4 = \frac{Y_{max} - Y_i}{Y_{max} - Y_{target}} \text{ for } Y_i > Y_{target}$$

d_4 = 1 for $Y_i < Y_{target}$

Where d_4 = the individual desirability of Viscosity at pH 6.0. The overall desirability values were calculated from the individual values by using the following equation:

$$D = (d_1 d_2 d_3 d_4)^{1/4}$$

RESULTS AND DISCUSSION:

A statistical model was used in order to estimate the relationship between the obtained responses and the independent variables. A stepwise multivariate linear regression was performed to evaluate the observations. Before application of the design, a number of preliminary trials were conducted to determine the control factors and their levels. The factors and their levels are shown in Table 2**.**

Table 2: Composition and Responses for 3^2 Factorial design for Ofloxacin

Batch code	X_1 g	X_2 g	Viscosity at pH 6.0 (cps)	Viscosity at pH 7.4 (cps)	Adhesiveness (Dyne/cm^2)	CPR after 8 hr (%w/v)	Overall Desirability $D = (d_1d_2d_3d_4)^{1/4}$
A1	-1	-1	227	20406	1274.8	85.53	0.00
A2	0	-1	875	21165	1363.1	84.45	0.00
A3	1	-1	1575	22867	1451.4	83.08	0.00
A4	-1	0	1778	34197	1912.2	83.50	0.83
A5	0	0	2591	35290	2059.3	82.87	0.85
A6	1	0	6508	36971	2353.6	79.7	0.82
A7	-1	1	15909	43627	2843.9	69.9	0.71
A8	0	1	31179	44112	3040.0	66.05	0.00
A9	1	1	39112	44978	3138.1	56.87	0.00

The statistical evaluation of the results was carried out by analysis of variance (ANOVA) using Microsoft Excel Version 2007. The ANOVA results (p value) of the effect of the variables on viscosity at pH 6.0 & pH 7.4, adhesiveness and percent drug release of *in situ* ophthalmic gel are shown in Table 3.

Table 3: Coefficient and p values for different evaluation parameters

Parameters	**Coefficients**					
	b_0	**b_1**	**b_2**	**b_{11}**	**b_{22}**	**b_{12}**
Viscosity at pH 6.0(cps)	4090.22	4880.16	13920.5	-696.93	11187.1	5463.75
p Value	0.1735*	0.03047	0.00158	0.7705*	0.0143	0.0384
R Square	0.9832					
Viscosity at pH 7.4(cps)	35273.56	1097.66	11379.83	318.7	-2626.83	-277.5
p Value	5.87×10^{-7}	0.0031	2.87×10^{-6}	0.2354*	0.0012	0.1658*
R Square	0.9996					
Adhesiveness (Dyne/cm^2)	2102.9	152.03	822.11	8.2	76.85	29.4
p Value	5.02×10^{-5}	0.019	0.000138	0.894*	0.26765*	0.5158*
R Square	0.9954					
CPR after 8 hr (%)	82.93	-3.2133	-10.04	-1.36	-7.71	-2.645
p Value	6.07×10^{-6}	0.01501	0.00055	0.3054*	0.0060	0.0427
R Square	0.9912					

*Regression coefficients, statistically insignificant (p>0.05)

The significant factors in the equations were selected using a stepwise forward and backward elimination for the calculation of regression analysis. The terms of full model having insignificant p value ($p>0.05$) have negligible contribution in obtaining dependent variables and thus are neglected. The equations representing the quantitative effect of the formulation variables on the viscosity at pH 6.0 & pH 7.4, adhesiveness and percent drug release after 8 hrs are shown below:

$$\text{Viscosity at pH 6.0 } (Y_1) = 4090.22+4880.16\ X_1+13920.5X_2-696.83X_1^2+11187.1X_2^2+5463.75X_1X_2 \quad (1)$$

$$\text{Viscosity at pH 7.4 } (Y_2) = 35273.56+1097.66X_1+11379.83X_2+318.66X_1^2-2626.83X_2^2-277.5X_1X_2 \quad (2)$$

$$\text{Adhesiveness } (Y_3) = 2102.9 + 152.03X_1 + 822.11\ X_2 + 8.2X_1^2 + 76.85X_2^2 + 29.4X_1X_2 \quad (3)$$

$$\text{CPR after 8 hr } (Y_4) = 82.93 - 3.2133\ X_1 - 10.04\ X_2 - 1.36X_1^2 - 7.71\ X_2^2 - 2.645\ X_1X_2 \quad (4)$$

Coefficients with one factor represented the effect of that particular factor, while the coefficients with more than one factor and those with second order terms represented the interaction between those factors and the quadratic nature of the phenomena, respectively. A positive sign in front of the terms indicated a positive effect, while a negative sign indicated a negative effect of the factors.

The equation 4 indicated that independent variable X_2 was strongly responsible for reduced percent drug release after 8 hours compared to independent variable X_1. A quadratic amount of independent variable X_2 showed more negative influence on percent drug release after 8 hours compared to quadratic amount of independent variable X_1. There were also interactions between two independent variables showed the negative influence on response. From the equations 1 to 4, it was concluded that Carbopol 974P had a positive effect on Viscosity at pH 6.0 & pH 7.4 and adhesiveness, while it had negative effect on % drug release. Noveon® AA-1 USP Polycarbophil had positive effect on Viscosity at ph 6.0 & 7.4 and adhesiveness, while it had negative effect on % drug release.

Desirability function was utilized to find out the best batch out of 9 batches. The result shown in Table 2 revealed that batch A5 was the best formulation since it showed highest overall desirability of 0.85. The values of the independent variables of batch A5 were considered as optimum values for the preparation of the *in situ* ophthalmic gel. Surface plots were obtained for the measured responses based on the model using Design Expert® software (STAT EASE, Demo version). The relationship between the independent variables and the responses could be explained further by using these surface plots. Figure 1- 5 showed the surface plots for viscosity

at pH 6.0 and pH 7.4, adhesiveness, percent drug release after 8 h and overall desirability as a function of factors X_1 (amount of carbopol 974P) and X_2 (amount of Noveon® AA-1 USP Polycarbophil). The surface plots indicated that the addition of polymers resulted in a higher viscosity at pH 6.0 & pH 7.4, higher adhesiveness and lower percentage drug release after 8 hrs and higher overall desirability. Increasing the amount of Noveon® AA-1 USP Polycarbophil resulted in lower % drug release because increase in amount of Noveon® AA-1 USP Polycarbophil resulted in increased viscosity of the gel which made the gel stiff and thereby decreased the drug diffusion rate.

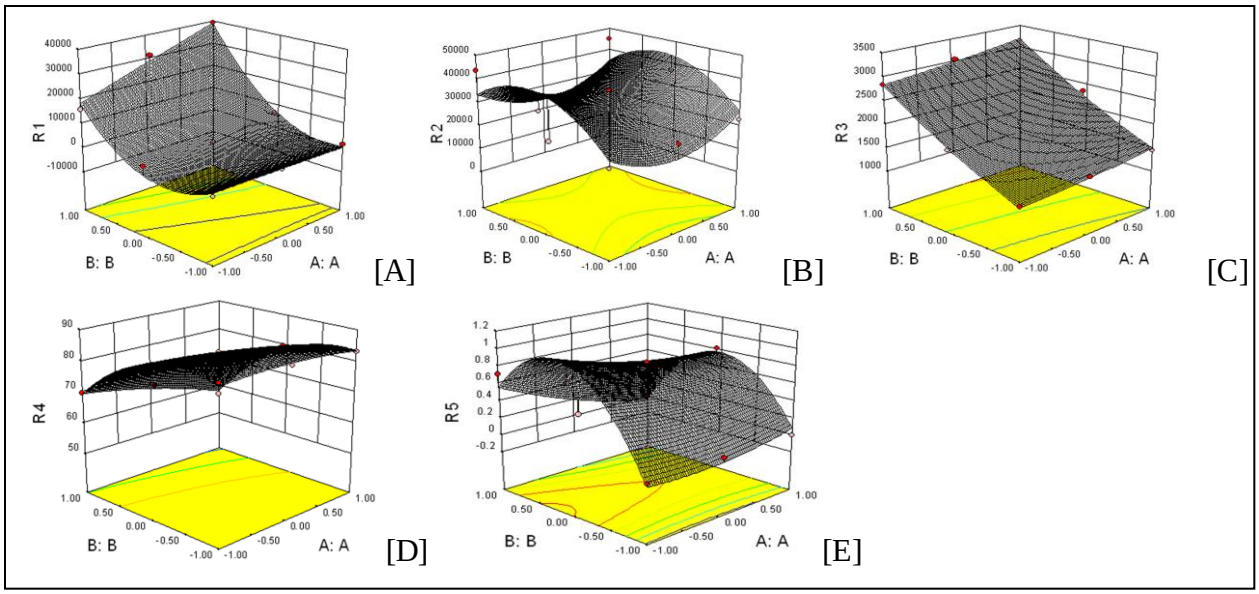

Figure 1: Surface plot showing relationship between

[A]: Viscosity at pH 6 (Y_1) and factor X_1 and X_2
[B]: Viscosity at pH 7.4 (Y_2) and factor X_1 and X_2
[C]: Adhesiveness (Y_3) and factor X_1 and X_2
[D]: Percentage drug release after 8 hrs (Y_4) and factor X_1 and X_2
[E]: Overall desirability value (Y_5)

It was observed that all the formulations underwent immediate transition into gel phase when came in contact with the simulated tear fluid (pH 7.4). The type of *in situ* gelling polymer, its concentration, and the type of bioadhesive polymer had a significant effect on the gelling capacity of ofloxacin formulations. The *in vitro* gelling capacity was found to be minimum in the formulation batches coded as A1, A2 & A3 while it was found maximum in the batches A4 to A9.(Table 4)

Adhesiveness

One important characteristic required for prolonged action is the ability of the formulation to be retained in the eye for longer period. The polymers used in this study had been described as

mucoadhesives. Therefore, they would be expected to show retention through formation of adhesive bonds with ocular tissues.

Table 4: Physical evaluation of *in situ* ophthalmic gels of Ofloxacin

Batch	pH	Drug content (%w/v)	Gelling capacity
A1	6.0	99.36	+*
A2	6.0	98.04	+
A3	6.01	98.46	+
A4	5.99	100.02	++
A5	6.0	98.92	++
A6	6.0	98.46	+++
A7	6.0	101.34	++
A8	6.02	98.04	+++
A9	6.0	98.26	+++

*+ Gelation occurred after a few minutes and gel dissolved rapidly,
++ Gelation immediate remains up to 8 hours,
+++ Gelation immediate remains more that 10 hours

The adhesiveness was presented in Figure. 2. In the method used to evaluate the mucoadhesive properties of the ofloxacin gel, the work required to over come the attractive forces developed between the surface of the sample and the surface of the disc was expressed as adhesiveness. The bioadhesive force was known to be dependent on the nature and the concentration of the bioadhesive polymer. In formulations consisting of Noveon® AA-1 USP Polycarbophil and Carbopol 974P, the adhesiveness of the gel increased as the concentration of each polymer increased. This might be attributed to the increased ability of these polymers to interact with the surface of the disc, but might also be a function of the increased tack of each formulation. Furthermore, the physical state of the polymeric component was observed to significantly affect the adhesiveness of the formulations. Hence, in the formulations where Carbopol 974P or Noveon® AA-1 USP Polycarbophil existed as suspended unswollen solids, the adhesiveness was greater than in the formulations in which these polymers exhibited a greater degree of swelling. The increased mass of unswollen particles in formulations containing Carbopol 974P 0.6%w/v compared to those containing Carbopol 974P 0.4% w/v or those containing Noveon® AA-1 USP Polycarbophil 0.75%w/v compared to those containing Noveon® AA-1 USP Polycarbophil 0.25%w/v has a direct effect on the adhesive strength. Keeping the concentration of Noveon® AA-1 USP Polycarbophil constant, the formulation containing 0.4%w/v of Carbopol 974P possessed lower adhesive force than formulation containing 0.5%w/v of Carbopol 974P. Thus, these results suggested that addition of viscosity enhancing agent to carbopol gel may improve its adhesiveness and other mechanical properties.

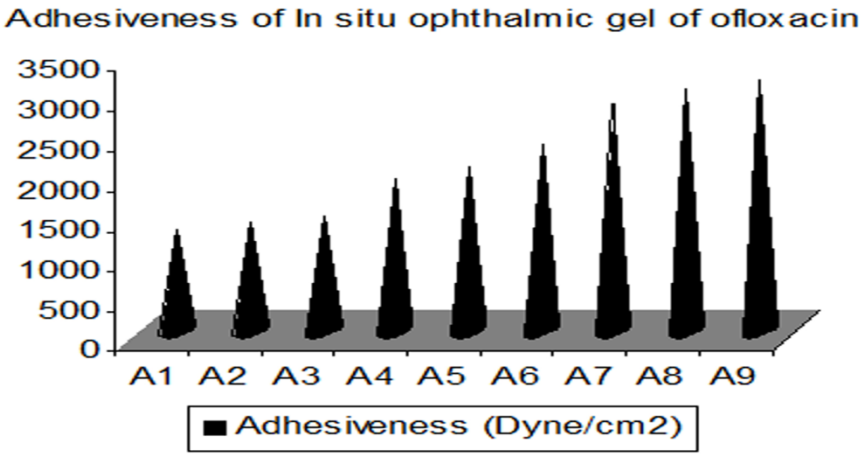

Figure 2: Comparative study of bioadhesion force of all the formulations.

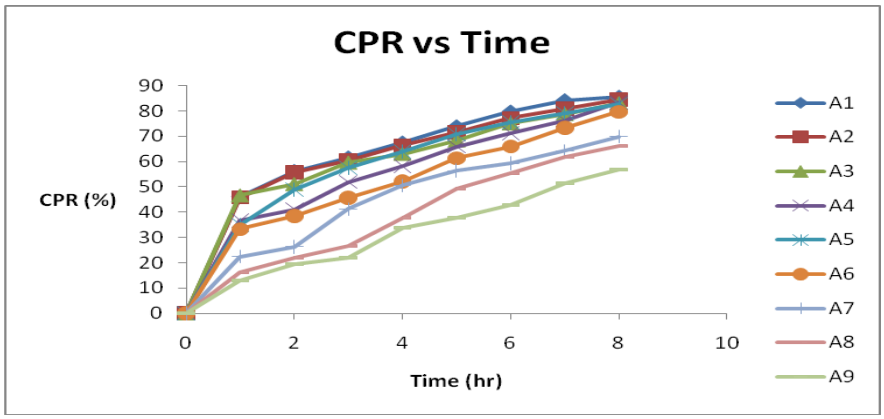

Figure 3: Comparative %CPR of Ofloxacin from the ophthalmic *in situ* gel.

***In vitro* drug release studies**

The comparison of *in vitro* drug release profiles are depicted in Figure 3. Formulations A7, A8 and A9 showed <25% drug release, while all the other formulations showed >25% drug release after one hour. It might be due to the interaction between two parameters i.e., the polymer type and its concentration. As the polymer concentration increased, the diffusion of ofloxacin through the formulation reduced due to more entangled nature of the polymeric network. In addition, the ingress of water into the formulation containing high concentration of polymer was reduced, thus lowering the rates of both dissolution/erosion. Also, the density of the chain structure of the gels increased at higher polymeric concentrations and this limit the active substance movement area. Finally, the degree of swelling increased as the concentration of the suspended solids increased, thus decreasing the diffusion of ofloxacin from the swollen matrix. The swelling phenomenon might be directly responsible for the effect of the type of polymer on the drug release from the formulation.

The *in vitro* drug release data were fitted to various kinetic models i.e., Korsemeyer-Peppas, Higuchi, Weibull, Zero and First order of release (Table 5 & 6). Results indicated that a Korsemeyer-Peppas matrix model was followed by all the batches. A faster release initially indicated that the drug in the solution in the space outside the gel matrix initially diffused quickly. The release of drug within the gel was controlled by both the nature and concentration of the polymers used.

Table 5: Model fitting of the drug release data (Korsemeyer-Peppas model)

Batches	K (kinetic constant)	n (Release exponent)
A1	3.879	0.2535
A2	3.865	0.2802
A3	3.785	0.2888
A4	3.491	0.4229
A5	3.611	0.3962
A6	3.413	0.4339
A7	3.010	0.6346
A8	2.679	0.6494
A9	2.484	0.7214

Table 6: Results of model fitting of optimized batch A5

Parameter	Korsmeyer-Peppas	Hixon Crowell	Weibull	Zero	First	Higuchi
SSR	2.0493	470.34	13.06	854.46	308.06	65.24
F value	0.3415	67.19	2.17	122.06	44	9.32
R square	0.999	0.95	0.992	0.845	0.981	0.988

Drug release was occurred by Fickian diffusion mechanism as reflected by its n value 0.396 ($n<0.5$) through the cornea with a pH triggered *in situ* gelling system containing bioadhesive polymer. It has been reported that as the *k* value increased, the release rate increased. Analysis of *k* value of the various formulations revealed that the release rate of ofloxacin was decreased as the concentration of each polymeric component was increased.

The formulations were exhibiting shear thinning system (pseudo plastic rheology) as an increased in shear stress was observed with increase in angular velocity (Figure 4- 6).

At pH 6.0, the formulations were in a liquid state and exhibited low viscosity. An increase in pH to 7.4 (the pH of the tear fluid) caused the solutions to transform into gels with high viscosity.

Antimicrobial efficacy studies

Zone of Inhibition of optimized batch and standard drug were found to be 1.8±0.2 and 2.1±0.2 respectively indicated that ofloxacin retained its antimicrobial efficacy when formulated as an *in situ* gelling system.

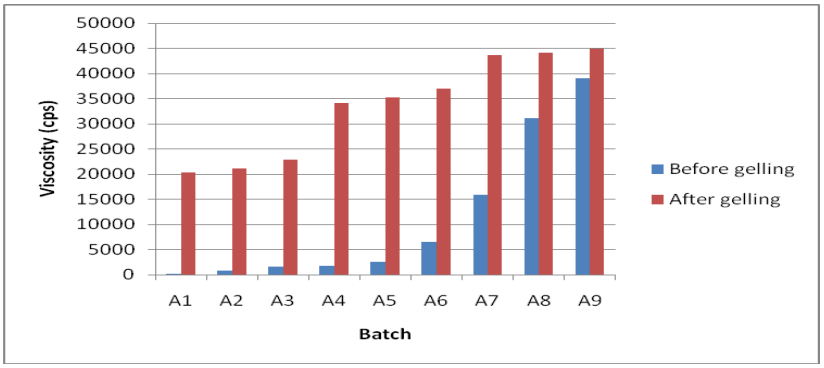

Figure 4: **Comparison of viscosity of the formulations before and after gelation**

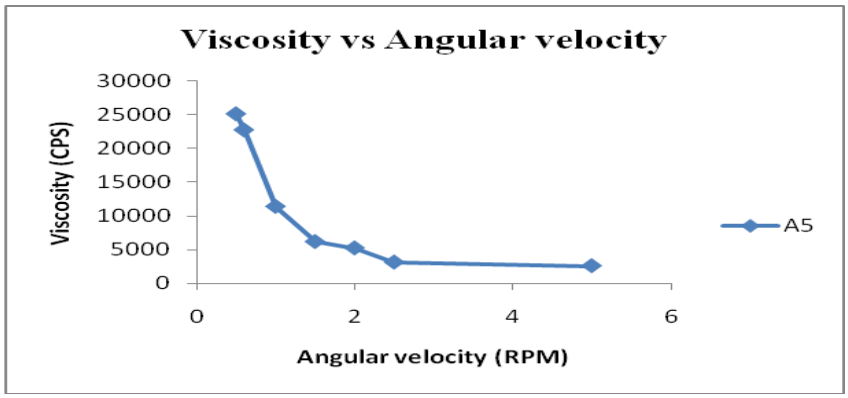

Figure 5: Rheological profile of *in situ* ophthalmic gel at pH 6.0

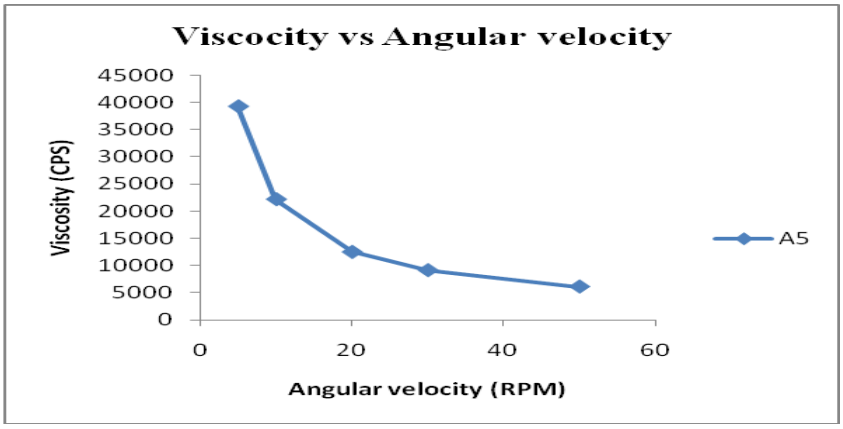

Figure 6: Rheological profile of *in situ* ophthalmic gel at pH7.4

Effect of sterilization

The autoclaving exerted insignificant effect on the drug content, viscosity and pH of the formulations. However, haziness was observed in all the formulations after autoclaving due to precipitation of the polymers. But, it was found to be disappeared and the original clarity was regained after overnight storage at ambient conditions.

Accelerated stability study

Study on optimized formulation batch A5 showed that the change was insignificant in various evaluation parameters i.e., clarity, pH, drug content, gelling capacity and viscosity at pH 6.0 & at pH 7.4 when the formulation at accelerated conditions.

CONCLUSION

Ofloxacin, a broad spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as pH triggered *in situ* gel forming eye drops (0.3% w/v) using Carbopol 974P as a gelling agent in combination with Noveon® AA-1 USP Polycarbophil as a viscosity enhancing agent. Thus, the developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance. Factorial experimental design, multiple regression analysis, contour plots, and desirability function have been proven to be a useful approach for the optimization of formulations.

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