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Full Length Research Paper

Design and characterization of ofloxacin mucoadhesive *in situ* hydrogel

Piyush B. Patel[1], Divyesh H. Shastri[1]*, Pragna K. Shelat[1], A. K. Shukla[1] and Gaurang B. Shah[2]

[1]Department of Pharmaceutics, K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, 382023, India.
[2]Department of Pharmacology, K. B. Institute of Pharmaceutical Education and Research, Gh-6 Road, Sector-23, Gandhinagar, Gujarat, 382023, India.

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The aim of the study is to design, optimize and characterize an ophthalmic *in situ* gelling system of an antibacterial agent, ofloxacin using polyacrylic acid (PAA) as the gelling agent along with Noveon® AA-1 USP Polycarbophil as a viscosity enhancer. A 3^2 full factorial design was applied for the optimization of the final formulation. The effect of independent variables was evaluated using dependent variables, that is, gel strength, bioadhesion force, viscosity and *in vitro* drug release profile of the formulation. Polynomial regression equations and surface plots were used to relate the dependent and independent variables. The desirability function was employed in order to determine the best batch which was then evaluated for an *in vivo* antimicrobial efficacy, effect of sterilization, ocular irritation and accelerated stability studies. From the factorial design, it was found that the optimum values of the responses could be obtained at medium levels of polyacrylic acid 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. Conclusively, 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* gel, ofloxacin, factorial design, poly(acrylic acid) (PAA), Noveon®AA-1 USP polycarbophil.

INTRODUCTION

Ophthalmic drug delivery system is extremely interesting and highly challenging endeavors (Ashim, 1993; Indu et al., 2004). The landscape of ophthalmic drug delivery is highly competitive and rapidly evolving. 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. The ocular bioavailability of drugs applied topically as eye drops is very poor. The absorption of drugs in the eye is limited by some protective mechanisms that ensure the proper functioning of eye and by other factors.

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 thereby reducing the systemic side effects. The sol-gel transition can be induced by a shift in the pH (Carbomer) (Saettone, 2002), temperature (Poloxamer) or by the presence of deacetylated gellan gum cations (Gelrite) (Reynolds, 1989; Sanzgiri et al., 1993; Cohen et al., 1997; Rozier et al., 1989).

The purpose of the study was to develop an *in situ* gelling ophthalmic delivery system of ofloxacin, a second generation fluoroquinolone used in external infections of

*Corresponding author. Email: divyeshshastri@gmail.com. Tel: (91) 9426420259.

the eye such as acute and sub acute conjunctivitis, bacterial keratitis and keratoconjunctivitis. The topical ophthalmic dose of ofloxacin is 1 to 2 drops of a 0.3%w/v solution in the affected eye(s) every 4 h in case of severe infection. A combination of poly (acrylic acid) (PAA) 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 provided by Bombay Tablet, Gandhinagar, PAA and Noveon® AA-1 USP Polycarbophil were supplied by Lubrizol. All the other materials used were of analytical grade.

Formulation design

The gel formulation was prepared aseptically by gradually dispersing the required amount of ofloxacin (0.3%w/v) and polymers (PAA/Noveon® AA-1 Polycarbophil) with continuous stirring for 1 h. Mannitol was added into the formulations as the osmotic agent. BKC solution (precold to <10°C) 0.01%w/v was added to obtain a final polymer concentration. All the samples were adjusted to pH 6.0±0.1 or 7.4±0.1 by 0.5 M NaOH solution and then stored in the refrigerator (approximately 24 h) prior to the evaluation. Sterilization was done by autoclaving at 121°C for 20 min.

Drug content uniformity study

The formulations were tested for content uniformity (Table 4). The vials (n = 3) containing formulation were properly shaken for 2 to 3 min. determined by dissolving an accurately weighed quantity of formulation (1 g in 50 ml) in pH 6.0 citro-phosphate buffer. The solution was filtered through 0.45 micron filter membrane and the drug concentration was determined by UV-Visible spectrophotometer at 288 nm.

In vitro gelling capacity study

The gelling capacity was determined by placing a drop of the system in a Petri dish containing 2 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 (Table 5). Time required for the gelation and the time taken for the formed gel to dissolve was taken as the evaluation parameters.

Rheological study

Rheological studies of the formulations were carried out using the Brookfield viscometer (Brookfield model R/S CPS, USA) (Gupta et al., 2010). The pH of the solutions was raised from 6.0 to 7.4 by neutralizing with 0.5 M NaOH, and simultaneously, the temperature of the solution was increased from 25 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

A modified device (modified Frantz diffusion cell using sheep cornea as a diffusion membrane) was used for evaluation of drug permeation. This membrane was tied to a specifically designed glass cylinder (open at both ends). Simulated tear fluid ($NaHCO_3$ 0.2 g, NaCl 0.67 g and $CaCl_2.2H_2O$ 0.008 g in 100 ml of water) was used as the diffusion medium. The formulation to be tested was added to the donor chamber with the help of a micropipette. The donor surface of the membrane was constantly in contact with the simulated tear fluid. A temperature of 37±0.5°C was maintained throughout the study. A magnetic stirrer to the cell provided continuous agitation. At regular time intervals, 5 ml of sample was withdrawn and replaced by fresh dissolution medium in order to maintain sink conditions to provide sink condition. The samples were diluted with the receptor medium and analyzed and the absorbance was measured at 288 nm using a UV-VIS spectrophotometer (Shimatzu UV-1800, Japan). Each experiment was performed in triplicate.

Cumulative drug release was calculated using equation generated from Beer Lamberts calibration curve in the linearity range of 0 to 10 µg/ ml. The release exponent 'n' and 'k' were calculated based on the Korsemeyer and Peppas model (Puglia et al., 2001).

Antimicrobial efficacy studies

Antimicrobial efficacy studies were carried out to ascertain the biological activity of sol-gel system against microorganisms. This was determined by agar diffusion test employing "cup plate technique" as per IP 1996. Both positive and negative controls were maintained throughout the study (Leung and Robinson, 1988).

Bioadhesion study

The prepared gel was evaluated as per the method described by Middleton et al. (1990). Excised sheep corneal membrane was immediately fixed with the mucosal side outwards onto a glass vial using a rubber band. Vials with the corneal membrane were stored at 37±0.5°C for 5 min. Then, the next vial with a section of membrane was connected to a balance in an inverted position, while the first vial was placed on a height adjustable pan. The gel was placed onto the corneal membrane of the first vial. Then, the height of the second vial was adjusted so that the membrane surfaces of both the vials came in close contact. A ten minute contact time was chosen. The weight was allowed to increase in the pan by adding water until the vials get detached. The bioadhesion force was measured as the minimum weight required for detaching two vials.

Effect of Sterilization study

The formulations were filled in 10 ml capacity amber glass vials, closed with grey butyl rubber closures and sealed with aluminium caps (Shastri et al., 2009, 2010a). The vials were subjected to terminal sterilization by autoclaving for 15 min. The formulations were evaluated for drug content, viscosity, clarity and pH before and after sterilization (n = 3).

Accelerated stability studies

Optimized formulation was placed in amber colored vial, sealed with aluminum cap and evaluated for a short term accelerated stability study as per ICH guidelines (Shastri et al., 2010b). Samples were

analyzed every month for drug content, clarity, pH, gelation temperature and rheological evaluation.

Factorial design

Optimization was done using 3^2 full factorial design in which two factors were evaluated and experimental trials were performed at all 9 possible combinations. In this design there are two independent variables and 3 levels (low, medium, and high) of each variable applied:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_1 \quad (1)$$

The amount of PAA as X_1 and Noveon®AA-1 USP Polycarbophil as X_2 were selected as independent variables (Table 1). The gel strength, bioadhesion force, viscosity (cps) at pH 6.0 and 7.4 and cumulative percent drug release were selected as dependent parameters. 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. DESIGN EXPERT 7.0.11 demo version software was used for formulation design.

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 previously, 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 h.

Bioadhesion 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 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:

$$D = (d_1d_2d_3d_4)^{1/4}$$

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.

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 and 7.4, adhesiveness and percent drug release of *in situ* ophthalmic gel are shown in Table 3. 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 and 7.4, adhesiveness and percent drug release after 8 h are shown as follows:

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

Table 1. Composition ofloxacin *in situ* gel formulation.

Ingredient (%)	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
PAA	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
Na_2HPO_4	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
BKC	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sterile water q. s. to	100	100	100	100	100	100	100	100	100

Independent variable	Levels		
	Low (-1)	Medium (0)	High (+1)
PAA (X_1) g	0.4	0.5	0.6
Noveon AA-1 USP Polycarbophil (X_2) g	0.25	0.50	0.75

Table 2. Experimental run using 3^2 full factorial design with desirability function.

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 at 8 h (%w/v)	Overall desirability $D = (d_1d_2d_3d_4)^{1/4}$
A1	-1	-1	227±15	20406±125	1274.8±25	85.53±1.25	0.00
A2	0	-1	875±20	21165±120	1363.1±14	84.45±1.15	0.00
A3	1	-1	1575±15	22867±115	1451.4±10	83.08±0.75	0.00
A4	-1	0	1778±48	34197±230	1912.2±15	83.50±1.84	0.83
A5	0	0	2591±38	35290±240	2059.3±20	82.87±0.56	0.85
A6	1	0	6508±85	36971±146	2353.6±25	79.7±1.65	0.82
A7	-1	1	15909±110	43627±176	2843.9±42	69.9±1.15	0.71
A8	0	1	31179±123	44112±180	3040.0±14	66.05±0.75	0.00
A9	1	1	39112±90	44978±315	3138.1±18	56.87±0.61	0.00

Table 3. Coefficient and p values for different evaluation parameters.

Parameter	Coefficient					
	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^2	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^2	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^2	0.9954					
CPR after 8 h (%)	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^2	0.9912					

*Regression coefficients, statistically insignificant ($p>0.05$).

Table 4. Physical evaluation of *in situ* ophthalmic gels of ofloxacin.

Batch code	pH	Drug content uniformity (%w/v)	*In vitro* gelling capacity
A1	6.0±0.02	99.36±1.14	+*
A2	6.0±0.01	98.04±1.06	+
A3	6.01±0.01	98.46±0.75	+
A4	5.99±0.02	100.02±0.45	++
A5	6.0±0.02	98.92±0.15	++
A6	6.0±0.03	98.46±1.84	+++
A7	6.0±0.01	101.34±2.05	++
A8	6.02±0.02	98.04±1.17	+++
A9	6.0±0.01	98.26±1.45	+++

*+, Gelation occurred after few minutes and gel dissolved rapidly; ++, gelation immediate remains for up to 8 h; +++, gelation immediate remains for more than 10 h.

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

Batch	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

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_1 (2)

Adhesiveness (Y_3) = 2102.9 + 152.03X_1 + 822.11 X_2 + 8.2X_1^2+ 76.85X_2^2 + 29.4X_1X_2 (3)

CPR after 8 h (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 (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.

Equation 4 indicated that independent variable X_2 was strongly responsible for reduced percent drug release after 8 h compared to independent variable X_1. A quadratic amount of independent variable X_2 showed more negative influence on percent drug release after 8 h compared to quadratic amount of independent variable X_1. There were also interactions between two independent variables which showed the negative influence on response. From Equations 1 to 4, it was concluded that PAA had a positive effect on viscosity at pH 6.0 and 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 and 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. The relationship between the independent variables and the responses could be explained further by using these surface plots. Figures 1 to 5 showed the surface plots for viscosity at pH 6.0 and 7.4, bioadhesion force, percent drug release at 8 h and overall desirability as a function of factors X_1 (amount of PAA) and X_2 (amount of Noveon® AA-1 USP Polycarbophil). The surface plots indicated that the

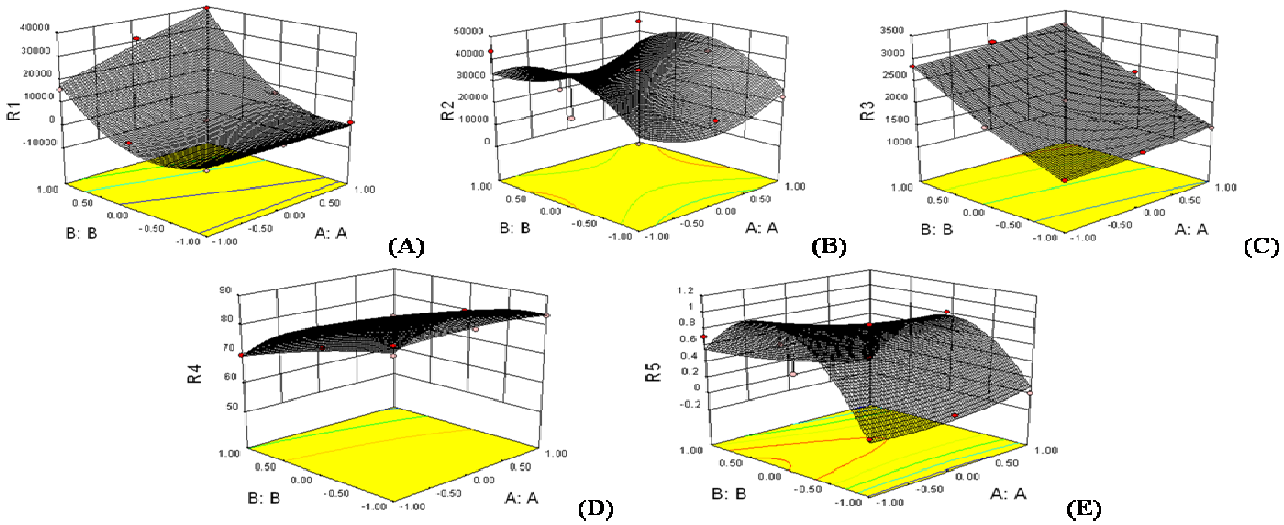

Figure 1. Surface plot showing relationship between; (A): Viscosity at pH 6 (Y_1) and factor X_1 and X_2 (B): Viscosity at pH 7.4 (Y_2) and factor X_1 and X_2; (C): Adhesiveness (Y_3) and factor X_1 and X_2; (D): Percentage drug release after 8 h (Y_4) and factor X_1 and X_2; (E): Overall desirability value (Y_5).

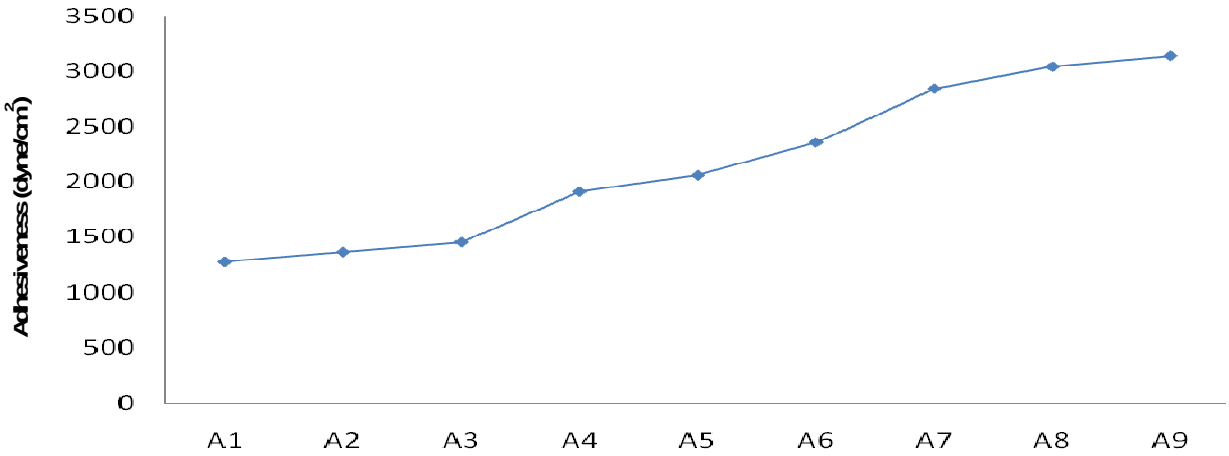

Figure 2. Comparative study of bioadhesion force of all the formulations.

addition of polymers resulted in a higher viscosity at pH 6.0 and 7.4, higher adhesiveness and lower percentage drug release after 8 h and higher overall desirability. Increasing the amount of Noveon® AA-1 USP Polycarbophil resulted in lower percent drug release because of increased viscosity of the gel which made the gel stiff and thereby decreased the drug diffusion rate.

It was observed that all the formulations underwent immediate transition into gel phase when they 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 and A3 while it was found maximum in the batches A4 to A9.

Bioadhesion study

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 affected significantly on adhesiveness of the formulations. Hence, in the formulations where PAA or

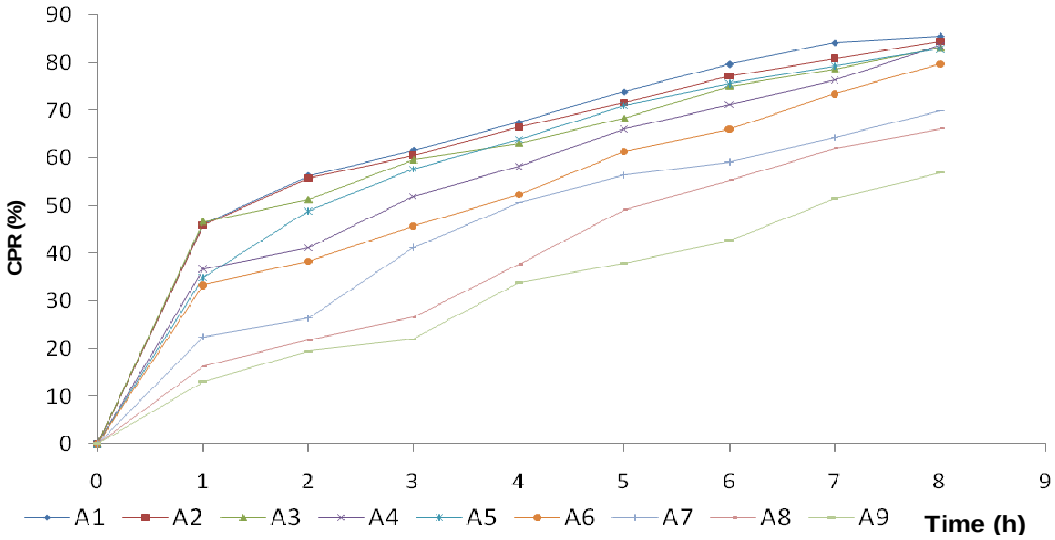

Figure 3. Comparative %CPR of ofloxacin from the ophthalmic *in situ* gel.

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^2	0.999	0.95	0.992	0.845	0.981	0.988

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 PAA 0.6%w/v compared to those containing PAA 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 PAA possessed lower bioadhesion force than formulation containing 0.5%w/v of PAA. Thus, these results suggested that addition of viscosity enhancing agent to PAA gel may improve its adhesiveness and other mechanical properties.

In vitro drug release studies

The comparison of *in vitro* drug release profiles are shown in Figure 3. Formulations A7, A8 and A9 showed <25% drug release, while all the other formulations showed >25% drug release at 1 h. This might be due to the interaction between two parameters, that is, 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, that is, Korsemeyer-Peppas, Higuchi, Weibull, Zero and First order of release (Table 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.

Drug release caused 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

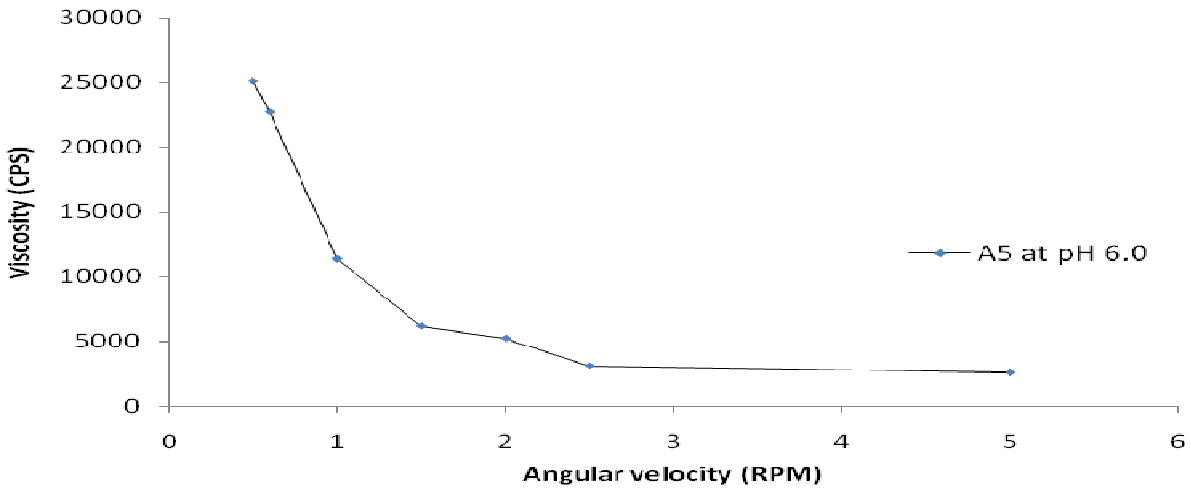

Figure 4. Rheological profile of *in situ* ophthalmic gel at pH 6.0.

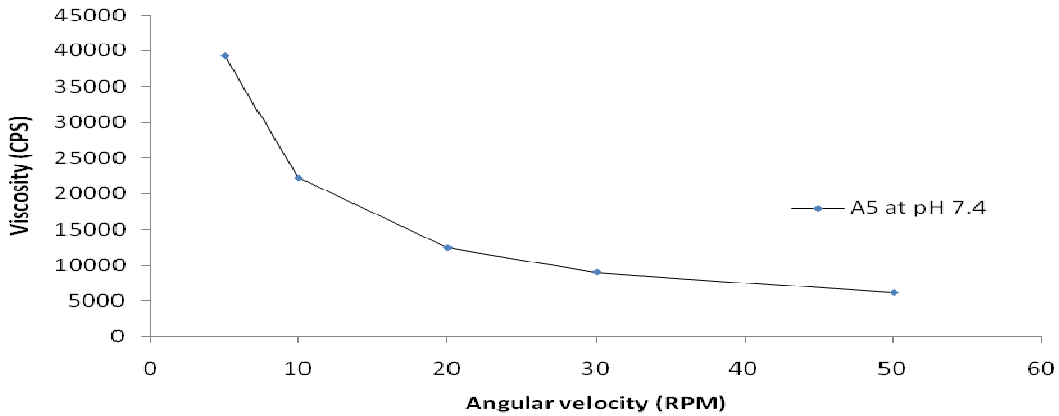

Figure 5. Rheological profile of *in situ* ophthalmic gel at pH 7.4.

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 (Figures 4 and 5).

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 indicating that ofloxacin retained its antimicrobial efficacy when formulated as an *in situ* gelling system.

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 have 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, that is, clarity, pH, drug content, gelling capacity and viscosity at pH 6.0 and pH 7.4 when the formulation is at accelerated conditions.

Conclusion

It was concluded that the amounts of PAA had significant effect on bioadhesion force and thereby drug release of the formulated *in situ* gel of ofloxacin. When It was used in combination with Noveon® AA-1 USP Polycarbophil for developing *in situ* ophthalmic drug delivery system of ofloxacin, low to moderate amount of PAA was to be used to achieve desired bioadhesion force, drug release profile and viscosity that is required in case of sustained ophthalmic drug delivery system.

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