Original Article

Use of factorial design in formulation and evaluation of ophthalmic gels of gatifloxacin: Comparison of different mucoadhesive polymers

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ABSTRACT: The aim of this research was to develop different ophthalmic gels of gatifloxacin using mucoadhesive polymers. To improve intraocular delivery of topically applied drugs such as gatifloxacin, gel formulations were prepared since solutions have a shorter ocular residence time because of tear turnover. A 3^2 factorial design was used to investigate the combined effect of two independent formulation variables in the preparation of the gels. Nine batches were prepared as per experimental design and evaluated for gelation temperature, gel strength, bioadhesion, viscosity, permeation, and antimicrobial efficacy. A surface plot was also created to graphically represent the effect of the independent variables on the evaluation parameters. Drug polymer compatibility was evaluated by differential scanning calorimetry and Fourier transform infrared spectroscopy. The prepared gels were observed to have a satisfactory gelation temperature, gel strength, and bioadhesion. Rheological study of the formulations indicated that gels exhibited pseudoplastic rheology. A modified device was used to evaluate drug permeation through a sheep's corneal membrane. *In vitro* permeation studies showed that a Peppas model was the best-fit model. Antimicrobial studies also indicated efficacy comparable to that of a marketed formulation. This systematic approach to formulation design should help in investigating the effect of variables in formulation processing.

Keywords: Factorial design, ophthalmic gels, gatifloxacin, poloxamer.

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1. Introduction

Ophthalmic delivery is one of the most interesting and challenging areas of pharmaceutical research (*1,2*). Topical ocular infections and especially fungal infections can be effectively treated with ocular delivery itself rather than with oral delivery of drugs. Eyedrops and suspensions are often used for topical administration of ophthalmically active drugs to tissues around the ocular cavity. A drawback of these preparations is that the active constituent present is diluted by tear film as the dosage form is introduced into the cul-de-sac and is rapidly drained away from pre-corneal cavity by constant tear flow and lacrimo-nasal drainage. As a result, only a small fraction of the dose is absorbed by ocular tissue. Hence, frequent administration and use of concentrated solutions appears to be a better approach to obtain the desired therapeutic effect (*3*). Some ocular delivery systems extend the duration of drug action by enhancing corneal absorption (*4*). These include soluble gels and emulsions (*5,6*), hydrophilic ocular inserts (*7*), ion-pair associations (*8*), and prodrugs and liposomes (*9-12*). The use of gels for the ocular administration of drugs offers many advantages compared to conventional eyedrops, mainly as a consequence of the more prolonged corneal contact time (*13*). Many techniques have been utilized to modify the response to drugs that are delivered topically to the eye. The concept of *in situ* forming gels using poloxamers has been reviewed by Karmarkar *et al.* (*14*) and such systems have been investigated using phase-transition polymers (*15-19*). Sodium alginate has been used extensively to form polymeric dispersions in buffers that typically show low viscosity up to pH 5 and coacervate in contact with tears and thus form gels (*20*). *In situ* gels of these polymers can be conveniently applied to the conjunctival sac where they undergo transition from a sol to gel. Prolongation of residence time due to these *in situ* gelling systems will help to deliver a drug continuously in a controlled manner to the anterior chamber of the eye and will eliminate frequent administration of the drug, thus leading to better patient compliance and extended action. This will result in a

dose reduction and help to minimize local and systemic side effects (*21*). The present work emphasizes the preparation and evaluation of various *in situ* gels of gatifloxacin prepared through use of sodium alginate and mucoadhesive polymers such as poloxamer 407, Carbopol 974P, and hydroxyethyl cellulose (HEC). A 3^2 factorial design was used to investigate the combined effect of two independent formulation variables in the preparation of *in situ* gels. A surface plot was also created to graphically represent the effect of independent variables on the evaluation parameters.

2. Materials and Methods

2.1. *Materials*

Gatifloxacin was donated by Cipla (Mumbai, India). Poloxamer 407 and Carbopol 974P were supplied by BASF (Schwarzheide, Germany) and Noveon (Mumbai, India), respectively. HEC was donated by Okasa Pharma (Maharashtra, India) and sodium alginate was purchased from Loba Chemie (Mumbai, India). All other chemicals were of analytical grade.

2.2. *Differential scanning calorimetry (DSC)*

Differential scanning calorimetry (DSC; SDT2960, TA Instruments Inc., New Castle, DE, USA) was performed using assess thermotropic properties and thermal behaviors of gatifloxacin, poloxamer 407, HEC, sodium alginate, a physical mixture of gatifloxacin/sodium alginate (1:1), Carbopol 974P, a physical mixture of gatifloxacin/sodium alginate/HEC (1:1:1), a physical mixture of gatifloxacin/poloxamer 407/HEC (1:1:1), a physical mixture of gatifloxacin/Carbopol 974P/HEC (1:1:1), and a physical mixture of gatifloxacin/poloxamer 407/Carbopol 974P/HEC (1:1:1:1). Samples (3-5 mg) were placed in aluminum pans with lids at a constant heating range of 15°C/min in a temperature range up to 300°C. Nitrogen was used as a purge gas through the DSC cell.

2.3. *Fourier transform infrared spectroscopy (FTIR)*

For Fourier transform infrared spectroscopy (FTIR), infrared spectra were obtained using a Perkin-Elmer Spectrum-one FTIR spectrometer (Shelton, CT, USA) with KBr disks. The samples (gatifloxacin, poloxamer 407, HEC, sodium alginate, Carbopol 974P, and their physical mixtures) were previously ground and mixed thoroughly with KBr. The KBr disks were prepared by compressing the powder. The scanning range was kept at 4,000-500 cm^{-1}.

2.4. *3^2 Factorial design and regression analysis*

Batches were prepared using a 3^2 factorial design (*22*,*23*). The advantages of a factorial design include greater precision. Using a factorial design allows examination of the effect of one variable when other factors are changed, something which is not possible using traditional methods of investigation. A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad \text{--- Eq. 1}$$

where Y is the dependent variable, β_0 is the arithmetic mean response of the nine runs, and β_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average results of changing one factor at a time from a low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. Multiple regression analysis and F statistics were used to identify statistically significant terms.

2.5. *Preparation of ophthalmic gels*

2.5.1. *Composition of poloxamer gel*

Formulations were prepared using a 3^2 factorial design (Tables 1 and 2). Different formulations of poloxamer 407 were prepared by the cold method

Table 1. Experimental design of poloxamer 407 gels

Variables	Level 1	Level 2	Level 3
Poloxamer 407 (%, w/w) (X_1)	18	20	22
HEC (%, w/w) (X_2)	1	2	3

Table 2. Compositions of ophthalmic gels using poloxamer

Formulation code	Gatifloxacin (%, w/w)	Poloxamer 407 (%, w/w)	HEC (%, w/w)	Tween 20 (%, w/w)	Benzalkonium chloride (%, w/w)
P1	0.3	18	1	2	0.01
P2	0.3	18	2	2	0.01
P3	0.3	18	3	2	0.01
P4	0.3	20	1	2	0.01
P5	0.3	20	2	2	0.01
P6	0.3	20	3	2	0.01
P7	0.3	22	1	2	0.01
P8	0.3	22	2	2	0.01
P9	0.3	22	3	2	0.01

(*24*). A calculated amount of poloxamer 407 was added to cold distilled water with continuous agitation using a magnetic stirring bar and then the required quantity of HEC was added with continuous stirring until it completely dissolved. The resulting dispersion was left at 4°C overnight until a clear solution was obtained. Gatifloxacin was dissolved in 0.1 N HCl and the solution was neutralized with 0.1 N NaOH. Benzalkonium chloride and Tween 20 were added to the above solution. The drug solution was added to the poloxamer solution. Volume was then brought up using phosphate buffer, pH 7.4. The developed formulations were placed in 10-mL amber glass vials closed with gray butyl rubber stoppers and sealed with aluminium caps. The formulations in their final form were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min.

2.5.2. *Composition of Carbopol 974P gels*

Formulations were prepared using a 3^2 factorial design (Tables 3 and 4). The detailed procedure for preparing the *in situ* gel-forming system of gatifloxacin is as follows: buffer salts were dissolved in 75 mL of purified water, and HEC was added and allowed to hydrate. Carbopol 974P was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer and Tween 20 was added with stirring. Gatifloxacin was dissolved in 0.1 N HCl solution and pH was adjusted with 0.1 N NaOH. Benzalkonium chloride was then added as a preservative. Volume was brought up using purified water. The developed formulations were placed in 10-mL amber glass vials closed with gray butyl rubber stoppers and sealed with aluminium caps. The formulations in their final form were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min.

Table 3. Experimental design of ophthalmic gels using Carbopol 974P

Variables	Level 1	Level 2	Level 3
Carbopol 974P (%, w/w) (X_1)	0.25	0.5	0.75
HEC (%, w/w) (X_2)	1	2	3

2.5.3. *Preparation of gels containing both poloxamer 407 and Carbopol 974P*

For preparation of poloxamer and Carbopol solutions (Tables 5 and 6), the already swelled Carbopol solution was cooled down to 4°C and the required amount of poloxamer 407 was added. The solutions were left at 4°C until a clear solution was obtained. Volume was brought up using phosphate buffer, pH 7.4. For preparation of drug-containing polymer solutions, gatifloxacin was dissolved in 0.1 N HCl solution, pH was adjusted with 0.1 N NaOH, and then this solution was added to the polymer solutions prepared as described above. Benzalkonium chloride was then added as a preservative. All of the sample solutions were adjusted to required pH values by 0.5 M NaOH solution and then stored in a refrigerator. The developed formulations were placed in 10-mL amber glass vials closed with gray butyl rubber stoppers and sealed with aluminum caps. The formulations in their final form were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min.

2.5.4. *Preparation of sodium alginate gels*

Formulations of gatifloxacin containing different concentrations of sodium alginate and HEC were prepared using a 3^2 factorial design (Tables 7 and 8). The ion-sensitive polymer, sodium alginate, was dissolved in 75 mL of phosphate buffer, pH 7.4. HEC was added with continuous stirring until it completely dissolved. Gatifloxacin was dissolved in 0.1 N HCl and the solution was neutralized with 0.1 N NaOH. Benzalkonium chloride was then added as a preservative to the above solution. Poloxamer 407 was added at a concentration of about 0.5% (w/w) to enhance the solubility of gatifloxacin. Phosphate buffer, pH 7.4, was then added to bring up the volume. The developed formulations were placed in 10-mL amber glass vials closed with gray butyl rubber stoppers and sealed with aluminium caps. The formulations in their final form were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min.

Table 4. Compositions of ophthalmic gels using Carbopol 974P

Formulation code	Gatifloxacin (%, w/w)	Carbopol 974P (%, w/w)	HEC (%, w/w)	Tween 20 (%, w/w)	Benzalkonium chloride (%, w/w)
C1	0.3	0.25	1	2	0.01
C2	0.3	0.25	2	2	0.01
C3	0.3	0.25	3	2	0.01
C4	0.3	0.5	1	2	0.01
C5	0.3	0.5	2	2	0.01
C6	0.3	0.5	3	2	0.01
C7	0.3	0.75	1	2	0.01
C8	0.3	0.75	2	2	0.01
C9	0.3	0.75	3	2	0.01

Table 5. Experimental design of ophthalmic gels using both poloxamer 407 and Carbopol 974P

Variables	Level 1	Level 2	Level 3
Poloxamer 407 (%, w/w) (X_1)	18	20	22
Carbopol 974P (%, w/w) (X_2)	0.1	0.2	0.3

Table 6. Compositions of ophthalmic gels using both poloxamer 407 and Carbopol 974P

Formulation code	Gatifloxacin (%, w/w)	Poloxamer 407 (%, w/w)	Carbopol 974P (%, w/w)	HEC (%, w/w)	Tween 20 (%, w/w)	Benzalkonium chloride (%, w/w)
PC1	0.3	18	0.1	1	2	0.01
PC2	0.3	18	0.1	1	2	0.01
PC3	0.3	18	0.1	1	2	0.01
PC4	0.3	20	0.2	2	2	0.01
PC5	0.3	20	0.2	2	2	0.01
PC6	0.3	20	0.2	2	2	0.01
PC7	0.3	22	0.3	3	2	0.01
PC8	0.3	22	0.3	3	2	0.01
PC9	0.3	22	0.3	3	2	0.01

Table 7. Experimental design of gels using sodium alginate gels

Variables	Level 1	Level 2	Level 3
Sodium alginate (%, w/w) (X_1)	1	2	3
HEC (%, w/w) (X_2)	1	2	3

Table 8. Composition of gels using sodium alginate

Formulation code	Gatifloxacin (%, w/w)	Sodium alginate (%, w/w)	HEC (%, w/w)	Poloxamer 407 (%, w/w)	Benzalkonium chloride (%, w/w)
S1	0.3	1	1	0.5	0.01
S2	0.3	2	1	0.5	0.01
S3	0.3	3	1	0.5	0.01
S4	0.3	1	2	0.5	0.01
S5	0.3	2	2	0.5	0.01
S6	0.3	3	2	0.5	0.01
S7	0.3	1	3	0.5	0.01
S8	0.3	2	3	0.5	0.01
S9	0.3	3	3	0.5	0.01

2.6. *Determination of gelation temperature*

Gelation temperatures of the gels were measured according to method described by Gilbert *et al*. (*25*). Two-mL aliquots of the gel were transferred to test tubes sealed with parafilm and immersed in a water bath at 4°C. The temperature of the bath was increased in increments of 1°C and left to equilibrate for 15 min at each new setting. The samples were examined for gelation, which was deemed to have occurred when the meniscus would no longer move upon tilting through 90°C. All measurements were performed in triplicate ($n = 3$).

2.7. *In vitro bioadhesion evaluations*

The bioadhesive force of all batches was determined by the method described by Choi *et al.* (*26*). A sheep's corneal membrane was cut from the eye of a sheep and instantly fixed with the mucosal side outwards onto a glass vial using a rubber band. Vials with the corneal membrane were stored at 37°C for 5 min. Then, the next vial with a section of membrane was connected to the balance in an inverted position while first vial was placed on a height-adjustable pan. The gel was placed onto the corneal membrane from the first vial. Then, the height of the second vial was adjusted so that the membrane surfaces of both vials would come in close contact. A contact time of 10 minutes was allotted. Then, the weight was allowed to increase in the pan until the vials detached. The bioadhesive force was the minimum weight required to detach two vials. The corneal membrane was changed for each measurement ($n = 3$).

2.8. *Determination of gel strength*

A sample of 50 g of gel was placed in a 100-mL graduated cylinder and gelled in a thermostat at 37°C. The apparatus for measuring gel strength as given by Choi *et al.* (*26*) was allowed to penetrate the gel. Gel strength, *i.e.*, the viscosity of the gels at physiological temperature, was determined by the time (in seconds) taken by the apparatus to sink down 5 cm through the prepared gel. All measurements were performed in triplicate ($n = 3$).

2.9. *Rheological studies*

Rheological studies were carried out using Brookfield's RVDV-II+ model viscometer (Brookfield Engineering Laboratories; Middleboro, MA, USA). The gel under study was placed in a small sample holder. Spindle LV 2 was used to measure viscosity. The viscosities were measured at room temperature and the speed of rotation of the spindle was increased from 10 to 200 rpm. Evaluations were conducted in triplicate ($n = 3$).

2.10. *Permeation studies across a sheep's corneal membrane*

A device designed by Gonjari *et al.* (*27*) was used to evaluate drug permeation through a sheep's corneal membrane. This membrane was tied to a specially designed glass cylinder (open at both ends). Simulated tear fluid ($NaHCO_3$ 0.218 g, NaCl 0.678 g, $CaCl_2 \cdot 2H_2O$ 0.0084 g, KCl 0.138 g in 100 mL of water) was used as a 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 simulated tear fluid. A temperature of 37 ± 0.5°C was maintained throughout the study. A magnetic stirrer in the cell provided continuous agitation. At regular time intervals, 1 mL of sample was withdrawn and replaced with fresh simulated tear fluid in order to maintain sink conditions. The samples were appropriately diluted and the absorbance was measured at 285.5 nm using a Shimadzu 1700UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan).

2.11. *Antimicrobial efficacy studies*

Antimicrobial efficacy studies were carried out by an agar diffusion test employing Bauer's well method (*21*). Sterile solutions of gatifloxacin (with a marketed eyedrop solution serving as standard solution) and the developed formulations (test solutions) were poured into wells bored into sterile nutrient agar previously seeded with test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*); after allowing diffusion of the solutions for 2 h, agar plates were incubated at 37°C for 24 h. The zone of inhibition (ZOI) measured around each well was compared to that of the control. The entire procedure except for incubation was carried out in a laminar airflow unit. Each solution was tested in triplicate. Both positive and negative controls were maintained through the study.

3. Results and Discussion

3.1. *Compatibility studies between drug and polymers*

DSC studies were carried out to determine interaction between the drug and polymers in the prepared ophthalmic gels. This will also indicate the success of stability studies (*28*). A gatifloxacin peak was clearly apparent in the DSC thermogram (Figure 1) in the form of a sharp characteristic endothermic peak in a temperature range of 182-185°C corresponding to its melting temperature (T_m). This showed that the gatifloxacin used was in pure form. A mixture of the drug and poloxamer 407 had no peak as poloxamer melted at 50°C, so there was no prominent peak resembling that of the drug. A mixture of the drug and Carbopol 974P and sodium alginate along with HEC showed endothermic peaks resembling the peak of the pure drug.

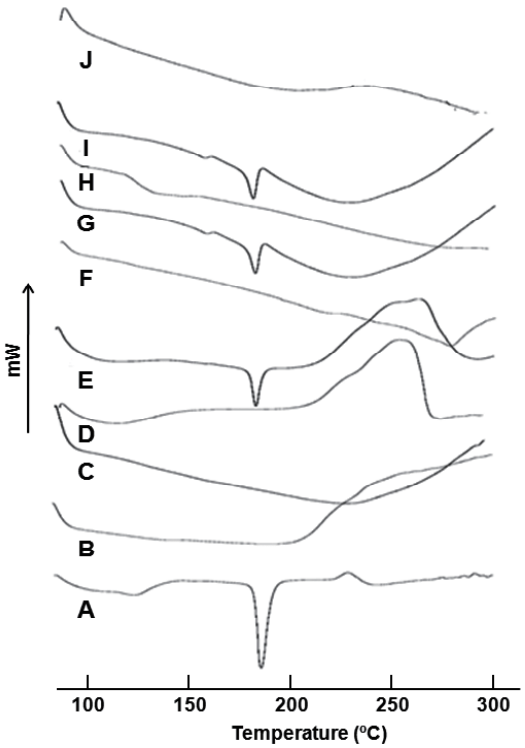

Figure 1. DSC thermograms of gatifloxacin and its mixtures with different polymers. A, gatifloxacin; **B**, poloxamer 407; **C**, HEC; **D**, sodium alginate; **E**, gatifloxacin/sodium alginate (1:1) physical mixture; **F**, Carbopol 974P; **G**, gatifloxacin/sodium alginate/HEC (1:1:1) physical mixture; **H**, gatifloxacin/poloxamer 407/HEC (1:1:1) physical mixture; **I**, gatifloxacin/Carbopol 974P/HEC (1:1:1) physical mixture; **J**, gatifloxacin/poloxamer 407/Carbopol 974P/HEC physical mixture.

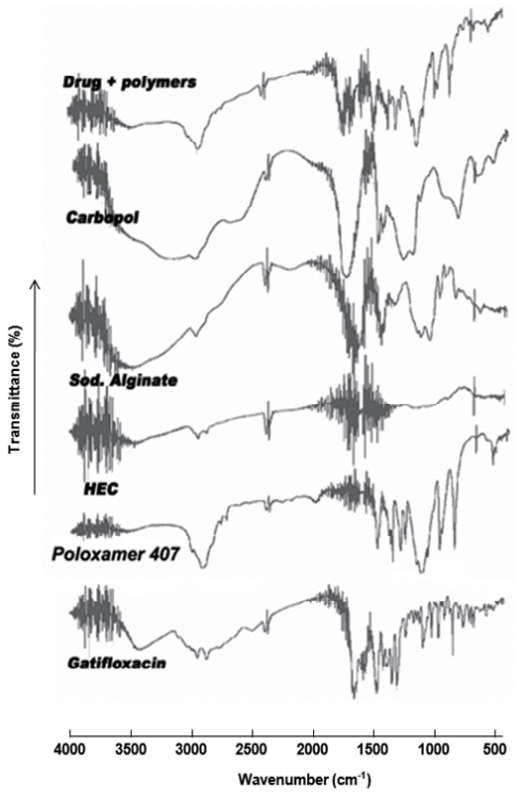

Figure 2. FTIR spectra of gatifloxacin, polymers, and their mixtures.

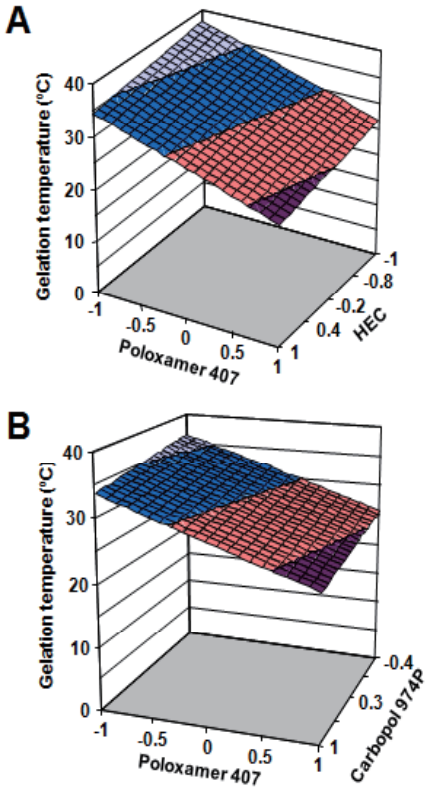

Figure 3. Effect of variables on gelation temperature. A, poloxamer 407 gels; **B**, poloxamer 407 and Carbopol 974P gels.

As shown in Figure 2, gatifloxacin's FTIR spectra showed characteristic peaks at 1,700.86 cm^{-1}, 1,507.44 cm^{-1}, 3,550.05 cm^{-1}, 3,408 cm^{-1}, 1,722 cm^{-1}, and 1,555cm^{-1}, respectively. These peaks were also mostly seen in the spectra of physical mixtures. There was only a slight shift in some of the groups, characteristic of the drug, poloxamer 407, and Carbopol 974P, that took place with overlapping and broadening of similar peaks. No new bands were detected in the spectra of physical mixtures, indicating no interaction between the drug and polymer mixture.

3.2. *Determination of gelation temperature*

Poloxamers were previously proven to undergo thermal gelation or sol-gel transition at a temperature of about 25 to 35°C. Below the transition temperature, poloxamer solutions allow a comfortable and precise delivery by the patient to the cul-de-sac, where thermogelation occurs. Immediate gelling increases a drug's residence time and enhances its bioavailability (*29*). Gels containing poloxamer 407 had good gelation properties in that the gelation temperature of the gel decreased as the concentration of poloxamer increased (Figure 3). This ability of poloxamer 407, a mucoadhesive polymer, to lower the gelation temperature may be due to increased viscosity after polymer dissolution and could be explained by the ability to bind to the polyoxyethylene chains present in the poloxamer 407 molecules. This would promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding, thus leading to gelation at lower temperature (*30*). A good degree of gelation was also shown by sodium alginate. Sodium alginate and Carbopol 974P did not have temperature-dependent gelling, so gelation could be determined only by gel strength and viscosity determination. The degree of gelation increased with an increasing concentration of sodium alginate. Out of all of the formulations, batches P1, P2, and PC1 (the composition of each batch is summarized in Tables 2 and 6) had significant *in vitro* gelation temperatures.

Table 9 summarizes the values of coefficients for the factorial equation. The coefficient of correlation was found to be about 0.98 for P and PC types of formulations. The negative values of β_2 indicate the ability of mucoadhesive polymers to lower the gelation temperature, which may be due to the reasons described previously.

3.3. *In vitro bioadhesion evaluations*

Bioadhesive force means the force with which gels bind to ocular mucosa. Greater bioadhesion is indicative of a prolonged residence time of a gel and thus prevents its drainage from the cul-de-sac. The addition of bioadhesive polymers HEC and Carbopol 974P increased the bioadhesive force (Figure 4). The bioadhesive force increased significantly as the concentration of bioadhesive polymers increased over

Table 9. Regression analysis of different evaluation parameters

Coefficients	Gelation temperature	*In vitro* bioadhesion	Gel strength	Permeation studies
Poloxamer gel				
β_1	–5.637*	836.0*	6.842*	–1.235*
B_2	–1.733*	152.9*	2.422*	–0.2443*
β_{11}	0.4563	213.8*	1.729*	–0.3385*
β_{22}	0.3875	–94.78*	0.267	–0.0760
β_{12}	0.2025	59.87*	0.406	–0.1069
R^2	0.9882*	0.9994*	0.9857*	0.9936*
F	50.35*	1073*	41.31*	93.22*
Carbopol 974 gel				
β_1	–	630.9*	16.32*	0.7033*
B_2	–	192.1*	4.873*	0.3983*
β_{11}	–	–56.83*	–5.653*	–0.2934
β_{22}	–	42.25	1.036	–0.3432*
β_{12}	–	53.10*	–1.624	–0.4296*
R^2	–	0.9976*	0.9825*	0.9463*
F	–	253.1*	33.60*	10.58*
Poloxamer 407/Carbopol 974P gel				
β_1	–5.429*	890.4*	6.842*	–1.370*
B_2	–1.493*	335.8*	2.422*	–0.2348*
β_{11}	0.2109	399.9*	1.729*	–0.1520
β_{22}	0.4083	13.69	0.267	–0.0239
β_{12}	–0.0453	88.85	0.406	–0.0834
R^2	0.9876*	0.9767*	0.9857*	0.9894*
F	47.83*	25.05*	41.31*	56.21*
Sodium alginate gel				
β_1	–	616.8*	3.252*	–1.140*
B_2	–	164.2*	1.493*	–0.4287*
β_{11}	–	213.8*	4.599*	0.2456
β_{22}	–	73.95	1.782	–0.1731
β_{12}	–	37.24	2.017*	0.3076
R^2	–	0.9836*	0.9525*	0.8879*
F	–	35.90*	12.03*	4.754*

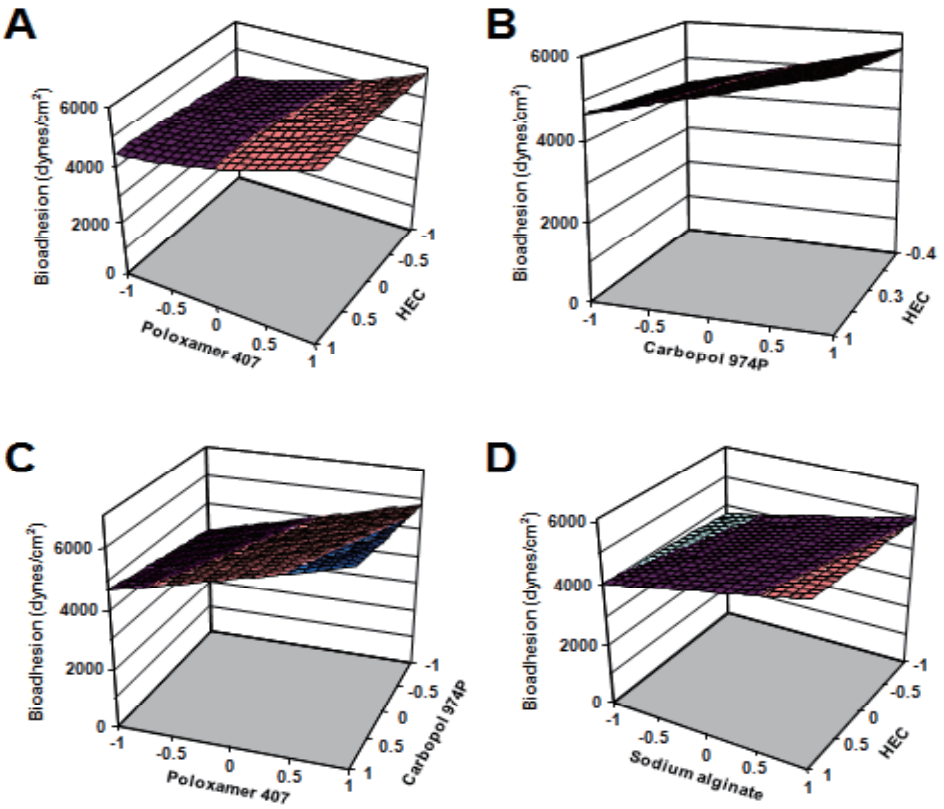

Figure 4. Effect of variables on bioadhesion. A, poloxamer 407 gels; **B**, Carbopol 974P gels; **C**, poloxamer 407/Carbopol 974P gels; **D**, sodium alginate gels.

the range of 1-3%. Bioadhesive forces of poloxamer solutions enhanced by the polymers used could be explained by the fact that secondary bond-forming groups (*e.g.* hydroxyl, ether oxygen, and amine) are the principal source of bioadhesion. Cellulosic polymers such as HEC have an abundance of hydroxyl and ether groups along their length (*31*).

Carbopol is known to be an excellent bioadhesive polymer (*32*). Hence, satisfactory bioadhesion was observed with all ophthalmic Carbopol gel formulations. The detachment stress and gel strength of gels of gatifloxacin were found to increase with the addition of Carbopol 974P (effect seen at high concentrations) (Figure 4). This increase was proportional to the concentration of Carbopol 974P. Efentakis *et al.* (*33*) reported that bioadhesion is determined by the availability of carboxyl groups. Carbopol 974P has a high percentage of carboxyl groups present. These groups simultaneously bind to the sugar residue in oligosaccharide chains present in the mucus membrane, resulting in a strong bond between the polymer and mucus membrane. The interaction increases with the increased density of hydrogen-bonding groups that interact with the glycoproteins of the mucin. Carbopol may also exhibit conformation, resulting in more favorable macromolecular accessibility of its functional groups for hydrogen bonding. Thus, the increase in the mucoadhesive force will lead to increased retention time and therefore increased bioavailability (*34*). In contrast, sodium alginate gels had less bioadhesion than poloxamer and Carbopol gels (Figure 4). However, increased bioadhesion was seen with an increase in the concentration of sodium alginate.

Factorial equation fitting and regression statistics (Table 9) for all types of formulations indicated good correlation coefficients. The interactive term X_1X_2 shows that both factors have a positive effect on bioadhesion.

3.4. *Determination of gel strength*

Gel strength provides an indication of the viscosity of gel formulations (*26*). An increase in gel strength was observed with all gel formulations. Specifically, gels containing Carbopol 974P had greater gel strength (Figure 5). This may be due to the reasons described previously. The coefficient of correlation (Table 9) was good. The use of mucoadhesive polymers such as HEC and Carbopol directly affects the gel strength, as indicated by positive β_2 coefficients.

3.5. *Rheological studies*

Rheological study of the formulations indicated that gels exhibited pseudo-plastic rheology, as evinced

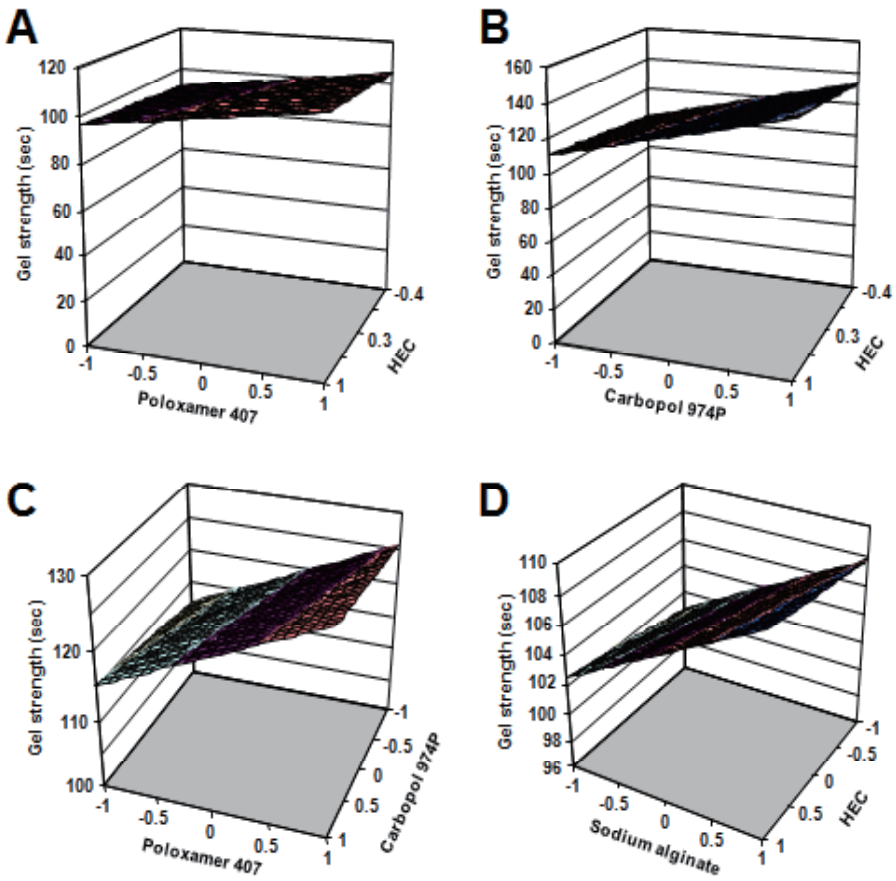

Figure 5. Effect of variables on gel strength. A, poloxamer 407 gels; **B**, Carbopol 974P gels; **C**, poloxamer 407/Carbopol 974P gels; **D**, sodium alginate gels.

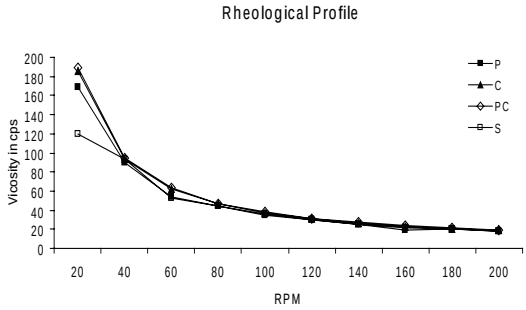

Figure 6. Rheological profile of different ophthalmic gels.

by shear thinning and a decrease in viscosity with increased angular velocity. As shown in Figure 6, viscosity for all formulations decreased in the order PC (poloxamer 407/Carbopol 974P gels) > C (Carbopol 974P gels) > P (poloxamer 407 gels) > S (sodium alginate gels). According to Bothner *et al.* (*35*), the administration of ophthalmic preparations should have as little effect as possible on the pseudo-plastic character of the precorneal film. The ocular shear rate is very high, ranging from 0.03 sec^{-1} during inter-blinking periods to 4,250-28,500 sec^{-1} during blinking (*36*), so preferred viscoelastic fluids often have a viscosity that is high under low shear rate conditions and one that is low under high shear rate conditions.

3.6. *Permeation studies across a sheep's corneal membrane*

In vitro permeation across a sheep's corneal membrane was fitted to various release kinetic models. All of the batches indicated that a Peppas model of permeation kinetics was the best-fit model. Initial faster release indicates that initially the drug in the solution quickly diffused in the space outside the gel. Release of the drug within the gel is controlled by the nature and the concentration of the polymer used.

As noted by Gonjari *et al.* (*27*), drug permeation through the cornea is greater with a thermoreversible gel containing HEC (Figures 7 and 8). The initial fast release of the drug from the formulations of gatifloxacin containing poloxamer 407 may be due to the rapid leaching of extramicellar ionized drug. Entrapped in the micelles, the drug may be released rather slowly. These findings correlate those reported by Paavola *et al.* (*37*), who suggested that drug diffusion from poloxamer 407 gel is through extramicellar aqueous channels and microviscosity.

Carbopol 974P gels showed an increase in the release of gatifloxacin, as compared to its release from poloxamer 407 and sodium alginate gels (Figures 7 and 8). HEC seemed to decrease the cumulative release. The retarding effect of the HEC could be attributed to its ability to increase the overall product viscosity (*38*) as well as its ability to distort or squeeze the extramicellar aqueous channels of poloxamer micelles through which the

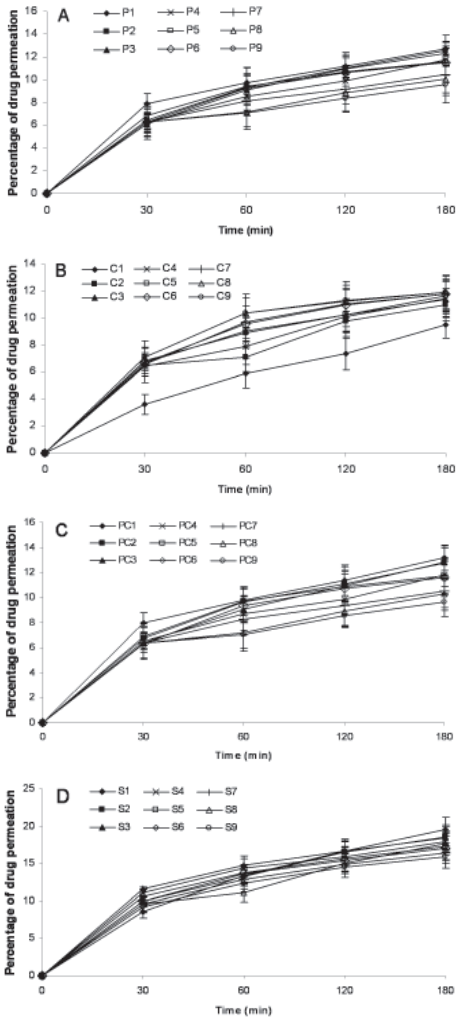

Figure 7. Drug permeation from ophthalmic gels. A, Drug permeation from poloxamer gels; **B**, drug permeation from Carbopol gels; **C**, drug permeation from poloxamer and Carbopol gels; **D**, drug permeation from sodium alginate gels.

drug diffuses, thereby delaying the release process (*39*). Carbopol 974P might lead to an increase in diffusion. This may be due to rapid swelling and hence faster diffusion of the drug. Electrostatic repulsion of the ionized carboxyl group may also result in decoiling and relaxation of the polymer network, leading to rapid dissolution of the drug and its fast release from the gel. An increased concentration of Carbopol 974P causes increased binding of Ca^{2+} binding sites and increased interaccessibility of Ca^{2+} binding sites causes relaxation of the polymer network (*40,41*).

A good coefficient of correlation (Table 9) was also noted. The interactive term X_1X_2 had a negative effect for P, C, and PC types of formulations. With the S type of formulations, the term $\beta_2X_2^2$ was found to be negative, indicating non-linearity that suggests that HEC might be responsible for decreasing permeation across the corneal membrane.

3.7. *Regression analysis*

Factorial equation fitting and regression analysis

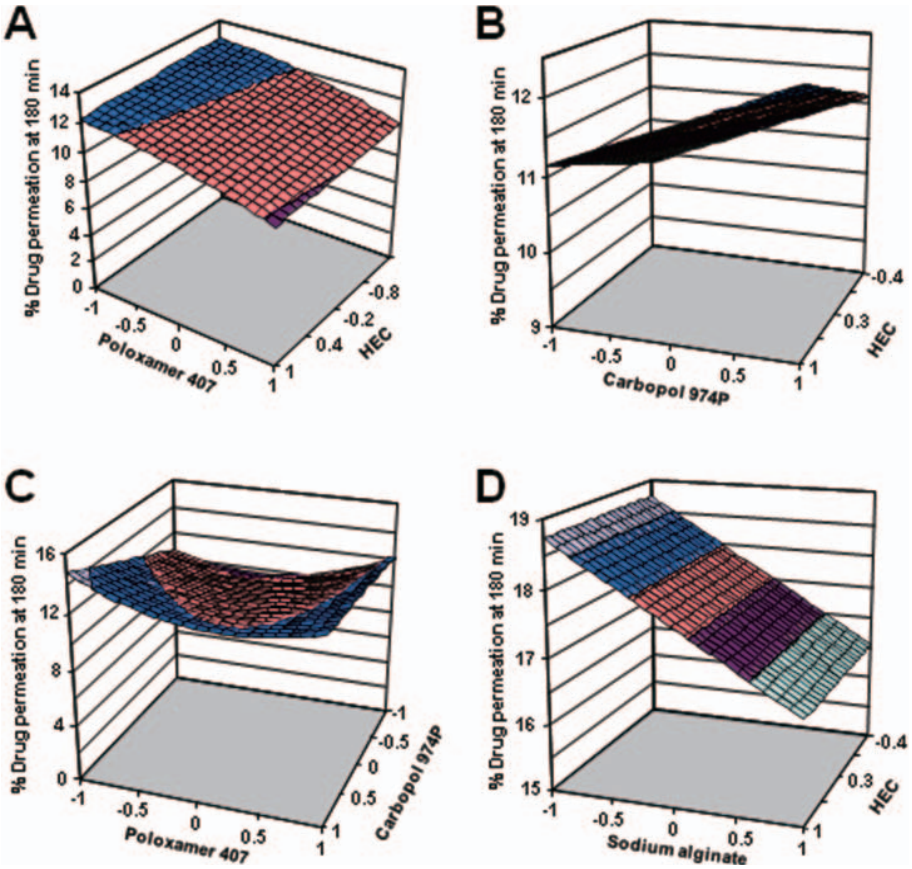

Figure 8. Effect of variables on percent drug permeation at 180 min. A, poloxamer 407 gels; **B**, Carbopol 974P gels; **C**, poloxamer 407/Carbopol 974P gels; **D**, sodium alginate gels.

Table 10. Antimicrobial efficacy studies of gels of Gatifloxacin

Sr. No	Formulation	Pseudomonas aeruginosa		Staphylococcus aureus	
		ZOI (cm)	% Efficacy	ZOI (cm)	% Efficacy
1	Standard	5.2	100	5.5	100
2	P	4.8	92.30	4.2	76.36
3	C	4.8	92.30	4.3	78.18
4	S	5.1	98.07	5.3	96.36
5	PC	5.0	96.15	5.1	98.07

of the different evaluation parameters for different formulations are shown in Table 9. The coefficients of the main effects (X_1 and X_2) and interactive terms (X_1X_2) were determined to indicate a change in the values of evaluation parameters when two factors were changed simultaneously. Multiple regression analysis and F statistics were used to identify statistically significant terms. Use of statistical methods has been found to allow greater discrimination among batches (*42*).

3.8. *Antimicrobial efficacy studies*

The formulations had satisfactory antimicrobial efficacy according to studies. Out of all four formulations, sodium alginate gels had maximum efficacy. The other three formulations also showed good antimicrobial efficacy, as indicated by zones of inhibition (Table 10, Figure 9).

4. Conclusion

In this study, *in situ* gelling ophthalmic gels of gatifloxacin were developed using mucoadhesive polymers. DSC and FTIR studies indicated compatibility between polymers and the drug. These gels were observed to have a satisfactory gelation temperature, gel strength, and bioadhesion. Rheological study of the formulations indicated that gels exhibited pseudo-plastic rheology, as shown by shear thinning and a decrease in the viscosity with increased angular velocity. *In vitro* permeation studies across corneal mucosa indicated that a Peppas model was the best-fit model. Antimicrobial studies indicated the effectiveness of these formulations. Therefore, these gels appear to be a viable alternative to conventional eyedrops. The results of 3^2 factorial experiments showed that

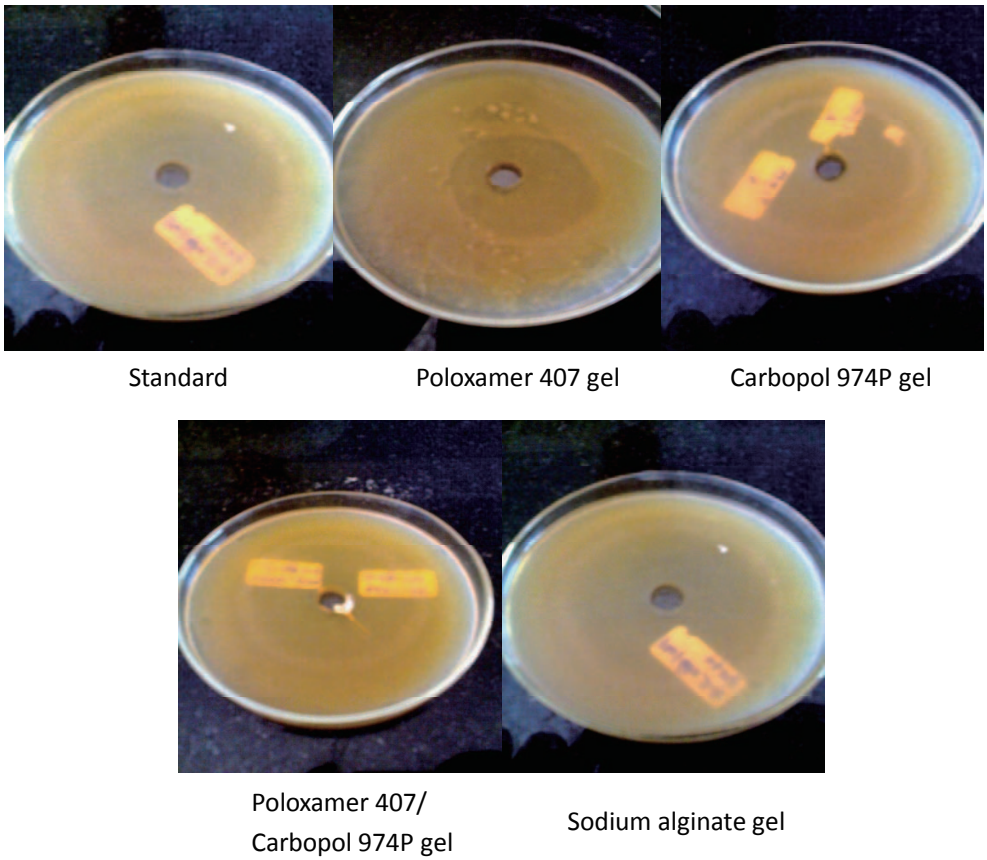

Figure 9. Zones of inhibition obtained during antimicrobial efficacy studies of gatifloxacin gels.

independent variables significantly affected dependent variables. The results obtained indicated that adopting a systematic approach can lead to an optimized formulation with fewer experimental requirements.

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