J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 47(1): 39-50, 2023
Doi: 10.33483/jfpau.1154051

ORIGINAL ARTICLE / ÖZGÜN MAKALE

DEVELOPMENT OF BESIFLOXACIN HCL LOADED OCULAR *IN SITU* GELS; *IN VITRO* CHARACTERIZATION STUDY

BESİFLOKSASİN HCL YÜKLÜ OKÜLER İN SİTU JELLERİN GELİŞTİRİLMESİ; İN VİTRO KARAKTERİZASYON ÇALIŞMASI

Heybet Kerem POLAT[1]*, Sedat ÜNAL[2]

[1]Republic of Turkey Ministry of Health, Turkish Medicines and Medical Devices Agency, 06100 Ankara, Turkey

[2]Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 38280, Kayseri, Turkey

ABSTRACT

Objective: *The aim of this study is to develop in situ gel formulations containing besifloxacin hydrochloride are heat triggered, which are prepared by using different poloxamer and derivatives different polymers that will change the gelling temperature to increase corneal contact time, regulate drug release, improve ocular bioavailability and increase patient compliance increase mucoadhesion.*

Material and Method: *Various concentrations of poloxamer 188 (P188) and poloxamer 407 (P407) were used to create the in situ forming gels. To increase the gel's capacity for bioadhesion, mucoadhesives such hydroxypropylmethyl cellulose (HPMC) or hydroxyethyl cellulose (HEC) were included in the formulations. Drug release in vitro, sol-gel transition temperature, rheological behavior, pH, clarity, and mucoadhesion force were all assessed for the produced formulations.*

Result and Discussion: *The developed formulations' gelation temperatures ranged from 29 to 35°C. The preparations' viscosity and mucoadhesion force increased with increasing P407, HPMC, and HEC concentrations. Besifloxacin HCl forms in situ gel formulas with K1, K2, K3, and K6 suited for mucoadhesion characteristics, gelation temperature, and viscosity. These formulations exhibit pseudoplastic flow. Increasing polymer concentrations resulted in a reduction in the burst release of the formulations. However, at the end of 6 hours, drug release was finished in all formulations. The results show that in situ gels containing P407 and P188 show promise for besifloxacin HCl application.*

Keywords: *Besifloxacin HCl, gel, in situ, ocular, poloxamer*

* **Corresponding Author / Sorumlu Yazar:** Heybet Kerem Polat
e-mail / e-posta: kerem_hybt@hotmail.com, **Phone / Tel.:** +903122183000

Submitted / Gönderilme : 03.08.2022
Accepted / Kabul : 19.10.2022
Published / Yayınlanma : 20.01.2023

ÖZ

Amaç: *Bu çalışmanın amacı, kornea temas süresi artırılmış, ilaç salımı düzenlenmiş, oküler biyoyararlanımı iyileştirilmiş ve hasta uyuncunu artırmak amacıyla jelleşme sıcaklığını değiştirecek farklı poloksomer türevlerinin ve mukoadhezyonu değiştirecek farklı polimerlerin kullanılması ile hazırlanan ısı ile tetiklenip jelleşen ve besifloksasin hidroklorür içeren in situ jel formülasyonları geliştirmektir.*

Gereç ve Yöntem: *İn situ jeller oluşturmak için poloksamer 188 (P188) ve poloksamer 407 (P407)'nin farklı farklı konsantrasyonları denenmiş bununla beraber in situ jelin biyo-yapışkanlık özelliklerini artırmak amacıyla formülasyonlara hidroksipropilmetil selüloz (HPMC) veya hidroksietil selüloz (HEC) gibi muko yapışkanlar ilave edilmiştir. Üretilen formülasyonların in vitro ilaç salımı, sol-jel geçiş sıcaklığı, reolojik davranışları, pH, berraklık ve mukoadezif kuvveti değerlendirilmiştir.*

Sonuç ve Tartışma: *Geliştirilen formülasyonların jelleşme sıcaklıkları 29 ila 35°C arasında olduğu belirlenmiştir. Preparatların viskozitesi ve mukoadezyon kuvveti, artan P407, HPMC ve HEC konsantrasyonları ile artmış. Patlama salımında bir azalma ile sonuçlanmıştır. Bununla beraber 6 saat sonunda bütün formülasyonlarda ilaç salımı bitmiştir. Jelleşme sıcaklığı, viskoziteleri ve mukoadhezyonları uygun olduğu için K1, K2, K3 ve K6 formülasyonları seçilmiş ve bu formülasyonlara besifloksasin HCl yüklenmiştir. Bu dört formülasyonun psödoplastik akış sergilediği tespit edilmiştir. Artan polimer konsantrasyonlarında formülasyonların patlama salımında bir azalma ile sonuçlanmıştır. Bununla beraber 6 saat sonunda bütün formülasyonlarda ilaç salımı bitmiştir. Sonuçlar, besifloksasin HCl uygulaması için P407 ve P188 içeren in situ jellerin umut vadettiğini göstermektedir.*

Anahtar Kelimeler: : *Besifloksacin HCl, in situ, jel, oküler, poloksamer*

INTRODUCTION

Because of the quick elimination caused by lachrymal flow, blinking, normal tear turnover, and solution drainage by gravity, conventional liquid ophthalmic administration systems have limited bioavailability and short precorneal residence times. To obtain the intended therapeutic effect, repeated solution instillation is therefore required [1].

A common and efficient method for treating bacterial conjunctivitis is topical antibacterial drug application to the conjunctival sac. The most popular antibiotics for treating bacterial conjunctivitis are fluoroquinolones (FQs), although resistance to FQs has emerged as with many other antibiotics. Besifloxacin, the newest fluoroquinolone, the first topical chlorofluoroquinolone, was created to have a stronger antibacterial effect because of its distinct molecular structure, specifically for the treatment of eye illnesses. Besifloxacin has proven its *in vitro* effectiveness against a number of infections, including those that are resistant to other FQs and antibiotic classes. It is applied to both gram positive and gram negative microorganisms. The U.S. Food and Drug Administration (FDA) approved besifloxacin suspension at 0.6% in 2009 for the treatment of bacterial conjunctivitis [2-4]. However, Besifloxacin HCl is a yellow powder with a molecular weight of 430.3 grams. Its melting temperature is 298°C. The PKa value is between 6-7. It has a water solubility of 0.08 mg/ml. As the pH of the medium gets closer to the acid, its solubility increases up to 10 mg/ml.

To extend the residence periods of the implanted dose and improve ocular bioavailability, a variety of ophthalmic vehicles have been created, including inserts, ointments, suspensions, and aqueous gels [5]. However, owing to several limitations, such as obscured vision from ointments or limited patient compliance from inserts, these ocular medication delivery systems have not been widely adopted [6].

To increase patient compliance, extend a drug's precorneal residence duration, and increase ocular bioavailability as a result, several *in situ* gelling systems have been developed. Due to a change in a particular physicochemical parameter (such as pH, temperature, and ions) in the cul-de-sac, these systems display sol-to-gel phase transitions [7].

Known for its great chemical compatibility, low toxicity, high solubility capacity for a variety of medications, and exceptional drug-release qualities, poloxamer is a surface-active block copolymer

composed of polyoxyethylene and polyoxypropylene [8,9]. However, because they may delay drug release and had a sufficient level of inertness for the eye, poloxamers have been widely used as an ocular drug delivery system. In contrast, a major drawback of poloxamer is its lack of mucoadhesive properties. As a result, additional polymers have been used to enhance the mucoadhesive properties of poloxamer-based ocular formulations, including sodium hyaluronate, carbopol, chitosan, hydroxyethyl cellulose (HEC), and hydroxypropylmethyl cellulose (HPMC) [10-12]. Ocular application of a thermosensitive *in situ* gel (prepared with p 407 and HPMC) loaded with ketorolac did not cause any irritation to the eye [13]. In the stability study performed with Doxycycline loaded thermosensitive *in situ* gels (prepared with P 408 and HPMC), it was determined that pH values, gelling temperatures and drug contents did not change at the end of the study [14].

Our study's goal was to create an *in situ* gelling ocular delivery system for 0.6% besifloxacin HCl employing combinations of poloxamer 407 (P407) and poloxamer 188 (P188). The gel bioadhesion characteristics of two mucoadhesives (HPMC or HEC) were optimized by adding poloxamer at various doses as an adjunct. The produced formulations were assessed for their pH, rheological behavior, sol-gel transition temperature, *in vitro* drug release, and clarity.

MATERIAL AND METHOD

Materials

Besifloxacin HCL (Molecular weight: 430.3g), poloxamer 407 (P407), poloxamer 188 (P188), hydroxypropyl methylcellulose (100 cp) (HPMC), hydroxyethyl cellulose (HEC) (640Cp), Benzalkonium chloride (BAK), sodium chloride and phosphate buffered saline (PBS) tablet were purchased from Sigma, Steinheim, Germany.

Preparation of the *In Situ* Forming Gels

For the creation of besifloxacin HCl *in situ* forming gels, various concentrations of P407 and P188 with mucoadhesives like HPMC and HEC were utilized. Weight-based preparation of medicated *in situ* forming gels was done utilizing the modified cold method [15]. Table 1 lists the ingredients in the prepared formulations.

In a nutshell, for formulations, P407 and P188 were progressively added to the predicted volume of cold acetate buffer (pH 4) (to increase the solubility of besifloxacin HCl) in a vial with a magnetic bar while being continuously mixed with a magnetic stirrer. (Labinco, model BV, The Netherlands). In order to create clear, homogeneous solutions, the partially dissolved poloxamer solutions were kept in the refrigerator and stirred occasionally. Additional quantities of the mucoadhesive polymers HPMC and HEC, each at concentrations of 0.5-1 percent (wt/wt), were mixed with the total poloxamer content during production. In all formulations, NaCl was used as an isotonicity agent and benzalkonium chloride as a preservative (Table 1).

pH

A pH meter was used to measure the pH. (HANNA, Germany). The measurements were made three times (n=3).

Clarity

After gelation, the *in situ* gel formulation's clarity was assessed by scrutinizing it in bright light against a dark background [16].

Gelation Temperature

The gelation temperature was measured according to the method reported by Qi et al. [17]. Briefly, each sample solution weighed 10 g, and it was put in a glass vial with a magnetic bar. The preparation was heated beginning at 20°C, increasing the temperature by 1°C/min, while stirring continuously at 100 rpm (Thermomac-TM19). It was decided to use the gelation temperature as the point at which the magnetic bar stopped moving. Three measurements were made for each.

Table 1. Ingredients in *in situ* gels

	Content of ingredients in each formulation (%, w/w)						
Code	**Poloxamer 407**	**Poloxamer 188**	**HPMC (LV)**	**HEC (HV)**	**BAK**	**NaCl**	**Acetate Buffer (qs)**
K1	15	10	0	0	0,001	0,2	100
K2	15	10	0,5	0	0,001	0,2	100
K3	15	10	0	0,5	0,001	0,2	100
K4	15	10	1	0	0,001	0,2	100
K5	15	10	0	1	0,001	0,2	100
K6	16	10	0	0	0,001	0,2	100
K7	16	10	0,5	0	0,001	0,2	100
K8	16	10	0	0,5	0,001	0,2	100
K9	16	10	1	0	0,001	0,2	100
K10	16	10	0	1	0,001	0,2	100

Viscosity

With a CP 52 spindle, the Brookfield, DV2T-RV Viscometer (Essex, UK) was used to measure the viscosity of *in situ* gels. The spindle runs at 1, 2.5, 5, 10, 20, 50 rpm angular velocity. *In situ* gel viscosities were assessed at their gelation temperature. At various angular velocities, viscosities were measured, and flow curves were created. Additionally, viscosity at 10 rpm was displayed for comparison. Three measurements were made for each.

Determination of The Mucoadhesive Force

Using sheep cornea tissue that was obtained locally from butchers, *in vitro* bioadhesive force was assessed using a texture analyzer instrument (TA.XT Plus, StableMicro Systems, UK) [3]. The eye was then situated on the lower arm of the texture analyzer after the *in situ* gel had been applied to one of the device's arms. For a contact period of 60 seconds, the probe from which the membrane was applied to the basis was reduced at a force of 0.2 N and a speed of 0.1 mm/s. Resistance to probe removal was tested to assess the *in situ* gel's bioadhesive efficacy. All bioadhesion measurements were performed in triplicate

Production of Besifloxacin HCl Contained *In Situ* Gel Formulation

All formulations were assessed for their physical characteristics, and an appropriate formulation was found. To a properly selected *in situ* gel formulation, besifloxacin HCl was added. These four formulations were named K1, K2, K3 and K6. Commercial formulations of besifloxacin HCl are available as 0.6% (w/v) ocular suspensions; consequently, besifloxacin HCl was employed *in situ* gel formulations at a drug concentration of 0.6 %. Table 2 lists the recorded formulations, together with Besifloxacin HCl and their physical characteristics.

Drug Content

Besifloxacin HCl concentrations were evaluated using a UV-vis spectrophotometer (UVmini-1240 Shimadzu Japan) at 289 nm after diluting 1 ml of *in situ* gel in 2 ml of water, followed by 1 hour of sonication in a bath sonicator [18].

$$\text{Drug Loading (\%)}=\frac{Amount\ to\ encapsulated\ MHL}{Total\ weighted}x100$$

Table 2. Physical characteristics of medication formulations and their constituents

Formulation components and Physical Properties	K1	K2	K3	K6
Besifloxacin HCl (% w/v)	0.6	0.6	0.6	0.6
Poloxamer 407	15	15	15	16
Poloxamer 188	10	10	10	10
HPMC	0	0,5	0	0
HEC	0	0	0,5	0
BAK	0.001	0.001	0.001	0.001
Sodium chloride	0.2	0.2	0.2	0.2
Acetate Buffer	100 ml	100 ml	100 ml	100 ml
pH (±SD)	4.02±0.01	4.05±0.02	4.01±0.03	4.03±0.02
Gelation temperature (°C±SD)	35±0.4	34±0.3	34±0.4	33±0.5
Viscosity (centipoise) 25°C	305±15	314±35	309±47	322±45
Viscosity (centipoise) 35°C	7642±124	8878±163	8745±212	10742±222
Clarity	Clear	Clear	Clear	Clear

In Vitro Release Studies

In situ gel formulations were tested for *in vitro* release using the dialysis bag method [19]. The dialysis membrans (Sigma, D9277) were closed, loaded with 100 µl of besifloxacin HCl, and then deposited at 37°C in 10 ml of an isotonic phosphate buffer at pH 7.4. In this way, the sink condition is provided. All of the medium were collected at various intervals (15, 30, 60, 120, 180, 240, and 360 minutes), and 10 ml new buffer media were transferred to replace the withdrawn samples. Besifloxacin HCl concentrations were determined by UV–vis spectrophotometer. The total amount of medicine released from each formulation over time was used to create a release profile for the besifloxacin HCl. Three duplicates of the experiment were carried out.

RESULT AND DISCUSSION

Characterization of *In Situ* Gel Formulations

The *in situ* gel formulations were made using a variety of P407 and P188 combinations with or without HPMC or HEC. Poloxamer-containing *in situ* gel compositions were transparent, clear, and colorless by visual inspection. Since non-transparent formulations may cause visual blur and are unpopular with patients, clarity is a highly desired property in ophthalmic formulations [20]. Examining the *in situ* gel formulations in this investigation, it was found that they were all clear (Table 3).

Using a pre-calibrated pH meter, the pH of *in situ* gel compositions was determined. All the formulations were found to have asidic pH, ranging between 4.00±0.03 to 4.07±0.01 (Table 1). To avoid causing any irritation at the application site and to stop the medicine from being washed away by the tears produced by the blink mechanism, the pH value of the ocular formulations should be the same as or close to the pH value of the eye. To maintain the stability of the active substance or improve its solubility, formulations with various pH levels can be created. Their solubility influences the bioavailability of medications, thus the items being prepared must dissolve more quickly and are made at a pH that won't irritate the eyes. However, because the preparations must be easily diluted with tears, the buffer strength utilized during preparation must be minimal. Examining the literature reveals that the pH range of 4 to 8 is suitable for eyes [21,22].

It has been determined that ophthalmic thermoreversible gels' sol-gel transition temperatures fall within the 25–34°C range, making them appropriate for ocular administration. If a thermosensitive formulation's gelation temperature is less than 25°C, a gel may develop at room temperature, and if it is

greater than 34°C, a liquid dosage form remains at corneal surface temperature, causing the formulation to drain from the eyes. Depending on the grade, concentration, and other formulation elements used, poloxamer solutions are reported to undergo thermoreversible gelation. Combining the two poloxamer grades would enable the gelation temperature to be modulated to fall within the acceptable range (25-34°C) [23].

Table 3. Results of *in situ* gels' *in vitro* characterization analysis

Formulation	pH (±SD)	Gelation temperature (°C±SD)	Viscosity (centipoise) 25°C	Viscosity (centipoise) 35°C	Clarity
K1	4.02±0.01	35±0.4	305±15	7642±124	Clear
K2	4.05±0.02	34±0.3	314±35	8878±163	Clear
K3	4.01±0.03	34±0.4	309±47	8745±212	Clear
K4	4.04±0.02	32±0.2	335±29	10252±128	Clear
K5	4.07±0.01	31±0.8	334±36	10548±132	Clear
K6	4.03±0.02	33±0.5	322±45	10742±222	Clear
K7	4.05±0.06	32±0.4	325±31	11642±432	Clear
K8	4.00±0.03	32±0.1	327±65	12742±467	Clear
K9	4.02±0.04	29±0.2	364±103	14813±424	Clear
K10	4.04±0.02	29±0.3	368±94	14942±214	Clear

To choose formulations with an appropriate sol-gel transition temperature and the lowest overall poloxamer concentrations, two combinations of polymers grades were investigated and used in the creation of the *in situ* forming gels. Results from formulations of besifloxacin HCl *in situ* forming gel consisting of P407/P188 (15/10 and 16/10 %, wt/wt) were excellent. All of the besifloxacin HCl *in situ* forming gel formulations were found to gel between 29 and 35°C, especially K1, K2, K3 and K6, which are thought to be acceptable for ocular administration. This is shown by the data in Table 3.

The inclusion of mucoadhesive polymers, which allow attachment of the formulations to the corneal mucin, would significantly minimize drainage of ophthalmic formulations from the precorneal surface. According to Table 3, the *in situ* forming gels' transition temperature gradually decreased as the concentration of the mucoadhesive polymers increased from 0.5 to 1%. The mucoadhesive polymers HPMC and HEC, which decreased the gelation temperature of the gels, were responsible for this. Such mucoadhesive polymers' capacity to bind to polyoxyethylene chains found in the poloxamer molecules may account for their ability to lower the gelation temperature. This will encourage dehydration, resulting in increased intermolecular hydrogen bonding and entanglement of nearby molecules, which will significantly boost gelation at lower temperatures [24].

Determination of the Mucoadhesive Force

For *in situ* forming of ophthalmic gels, the mucoadhesive force is a crucial physicochemical factor because it delays the formulation's precorneal residence time by preventing fast drainage. Fig 1 shows the findings from the analysis of the mucoadhesive forces of all the developed formulae.

In formulations comprising mucoadhesive polymers as HPMC and HEC, the prepared besiloxacin HCl *in situ* forming gels' mucoadhesive force dramatically increased. The mucoadhesive polymer concentration in the formulations was also increased, which significantly increased the mucoadhesive force. This could be explained by the fact that the primary source of mucoadhesion is secondary bond forming groups, such as carboxyl, hydroxyl, ether, oxygen, and amine, and that the cellulosic polymers used to make the *in situ* forming gels have a lot of hydroxyl and ether groups along their length, which are in charge of the mucoadhesive properties. The bonds-forming groups in the *in situ* forming gels rose as the concentration of cellulosic derivatives increased, improving the formulations' ability to adhere to mucous membranes.

The mucoadhesive forces of formulations made with HEC are larger than those of formulations having the same proportion of HPMC, according to a closer examination of the mucoadhesive forces in Fig. 1. This might be explained by the use of HEC rather than HPMC, which has a higher viscosity and molecular weight. To enhance adhesion through entanglements and van der Waals forces, high molecular weight is crucial [25].

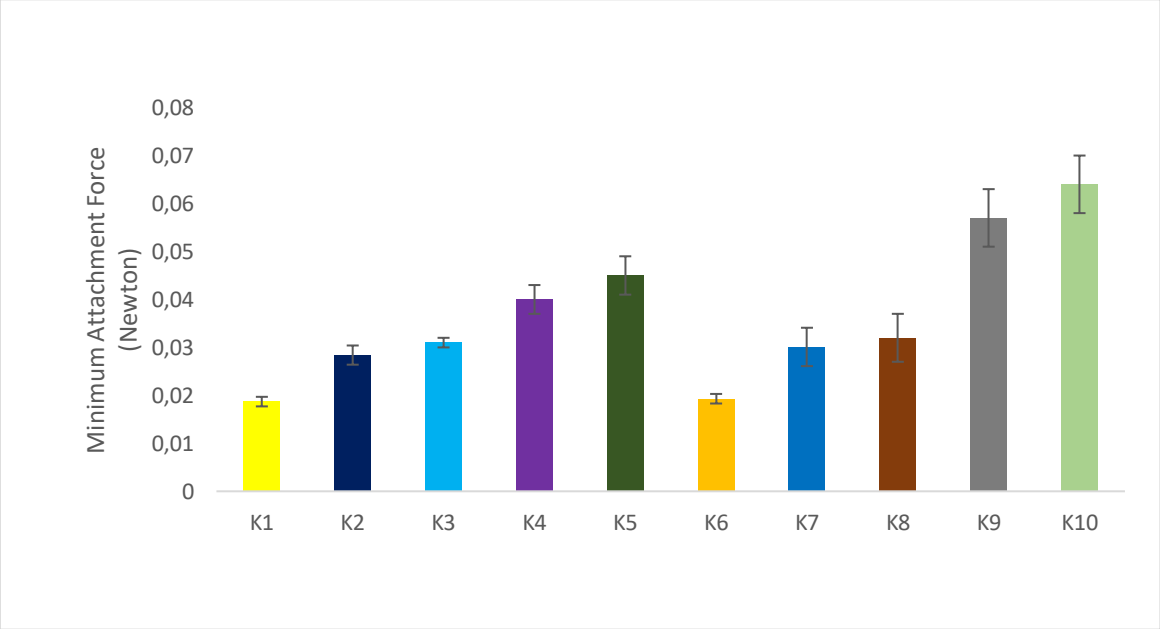

Figure 1. Mucoadhesion study results for formulations

Viscosity

The effects and usefulness of *in situ* gels *in vivo* are improved by rheological characteristics. When using extremely viscous *in situ* gels, a variety of issues could occur, however when using low viscosity *in situ* gels, tears would quickly remove them from the eye's surface. High viscosity ocular formulations are undesirable because they frequently leave a noticeable residue on the side of the eyelid where they are applied. It is possible to lessen the deleterious effects of ocular reflexes, such as blinking, on the formulation by using *in situ* gel formulations with pseudoplastic behavior. Longer corneal contact time and a more comfortable application are made possible by the formulation's pseudoplastic flow [26].

The impact of rotational velocity modifications on the viscosity of a chosen formulation is depicted in Figure 2 All *in situ* gel formulations at respective gelling temperatures showed pseudoplastic flow (shear thinning system) similar to tear fluid when the rheological characteristics were studied.

According to a review of the literature, the ideal viscosity for optimal ocular administration is between 50 and 50,000 cp. The bioavailability will rise with increasing viscosity as the formulation's time spent in the cul de sac region of the eye rises [14]. As a result, viscosity values for all formulations were determined at 10 rpm at both 25°C and 35°C. The evaluation of the results revealed that the viscosity values varied according to the polymer concentrations (Table 3). This example demonstrates the significant impact that polymer concentration has on viscosity. Examining the literature revealed that the findings were consistent [27].

Four formulations were chosen after the characterization research of all *in situ* gel formulations (pH value, gelation temperature, clarity, viscosity, and mucoadhesion). In each of the four formulations, 0.6 percent of besifloxacin HCl was added (Table 2). The formulas in question are K1, K2, K3, and K6. All formulations were found to be transparent, with pH values around 4, and have gelling temperatures between 35 and 33°C.

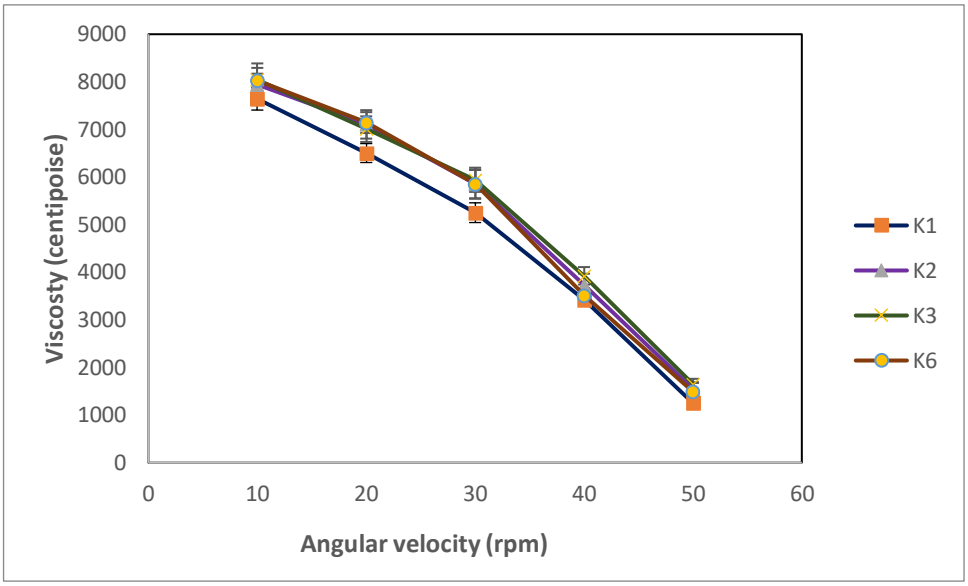

Figure 2. Rhelogical behaviors of *in situ* gelling systems

Drug Loading

It has been discovered that all formulations have loading capacities of more than 97 % (Fig 3). This demonstrates that medication loading is unaffected by polymer concentration. Examining the literature reveals that the findings are consistent [5,28].

Drug Release

Formulations containing besifloxacin HCl (percent 0.6) were employed, and *in situ* gels were subjected to *in vitro* drug release tests at 35°C and pH 7.4 isotonic phosphate buffer. The *in vitro* release profiles of besifloxacin HCl are shown in Figure 4. Examining the release patterns of the formulations K1, K2, and K3 for besiloxacin HCl revealed that some produced > 65 percent drug release, whereas K6 showed 58 percent release for two hours. The besifloxacin HCl had released 95% by the end of the sixth hour. As a result, the sixth hour was considered to be the last time the drug was released.

The findings demonstrate that the amount of medication burst released was lowered as poloxamer P407 concentration rose from 15 to 16%. These findings show that when the concentration of P407 rose, the gel's structure acted as a more formidable barrier to drug release. The decrease in the number and size of water channels and the rise in the number and size of micelles inside the gel structure may be the mechanisms causing this improved resistance [29]. Higher viscosity and a slower rate of drug release are a result of more cross-links between surrounding micelles due to the smaller intermicellar distance [30]. The rheology investigation that shows a direct correlation between gel concentration and viscosity may strengthen this idea [31].

The findings demonstrate that the amount of medication released was lowered as poloxamer P407 concentration rose When polymer in the formulation is involved, analysis and modeling of medication releases become challenging. *In situ* gels are created using polymers that expand in water, such as poloxamer, HPMC, and HEC. These polymers release through a mechanism controlled by diffusion, erosion, or a combination of the two [32]. Utilizing the non-linear regression model of KinetDS, the drug release kinetics of besifloxacin HCl from *in situ* gels were determined. Table 4 lists the parameters and R2 determination coefficients (determination coefficients) that were determined using this method. Evaluation in the study was done using a variety of models, including zero-order, first-order, Hixson-Crowell, Higuchi, Weibull, and Korsmeyer-Peppas models. The release kinetics of besifloxacin HCl in all formulations follow the model established by Weibull when the regression coefficients are examined. The release data from swellable polymeric systems best fit the Weibull model, according to certain research that has been studied in the literature [33-35].

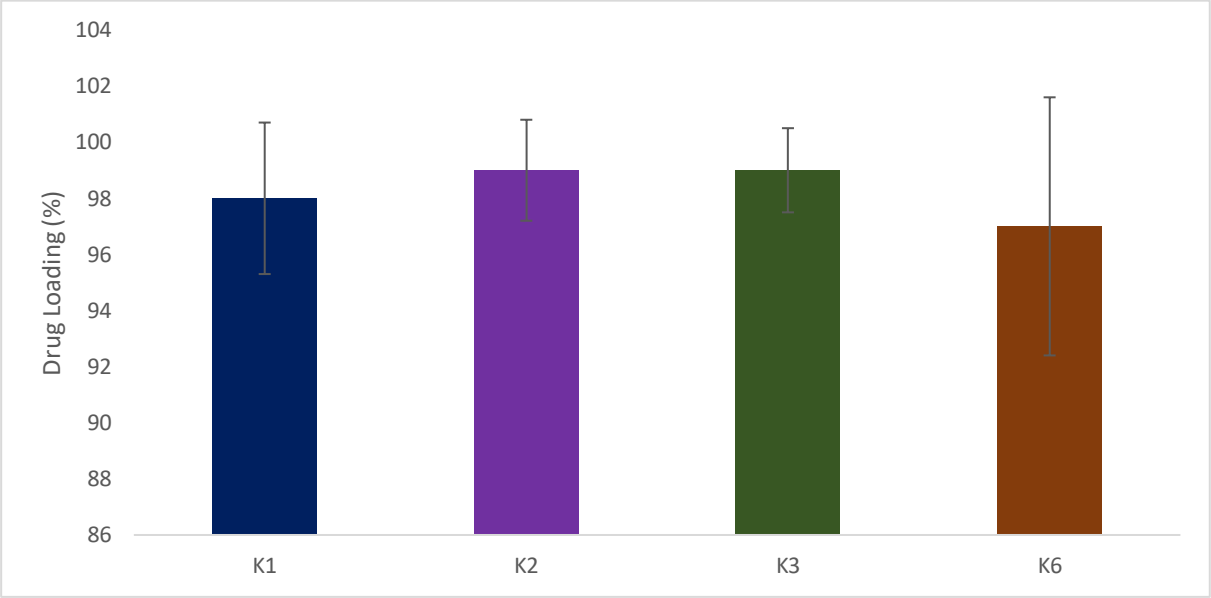

Figure 3. Drug loading given in percentages for Besifloxacin HCl (n=3)

Table 4. *In vitro* release kinetic parameters of besifloxacin from *in situ* gel

Sample	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Korsmeyer-Peppas (R^2)	Weibull (R^2)	Hickson-Crowell (R^2)
K1	0.8144	0.6880	0.7215	0.9618	0.9854	0.7351
K2	0.8151	0.6902	0.8007	0.9482	0.9931	0.7266
K3	0.8042	0.6826	0.7984	0.9621	0.9954	0.7232
K6	0.8002	0.6571	0.7975	0.9447	0.9889	0.7042

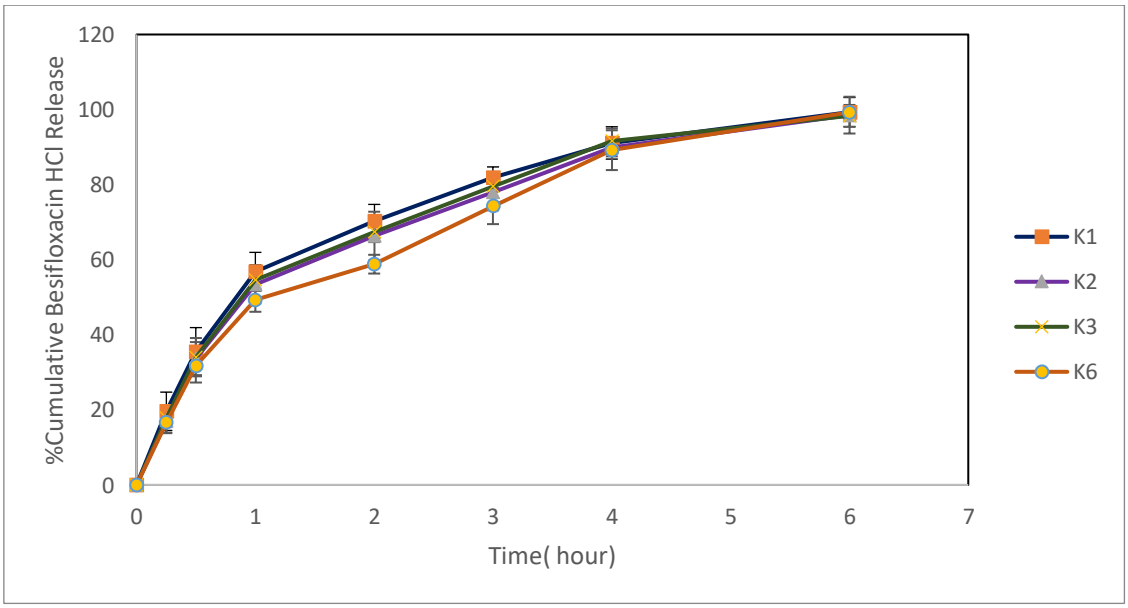

Figure 4. Cumulative release of besifloxacin HCl from *in situ* gel

In conclusion**,** as part of the investigation, multiple polymer solutions were made that had different concentrations of P407, P188, and the mucoadhesive polymers HPMC and HEC. All of these formulations underwent *in vitro* evaluation (pH, clarity, gelation temperatures, rheological behavior, and mucoadhesive tests). All formulations were discovered to exhibit a pseudoplastic flow resembling tears. The polymer ratios employed affect the corneal adhesion of each formulation, yet it can be seen that they are all at the intended level. When the gelation temperatures were compared to the poloxamer amounts, it was discovered that the gelation temperature dropped. K1, K2, K3, and K6 formulas were chosen because they gel at eye temperature. However, it was discovered that the pH of all formulations was close to 4. Since the pH range for ocular administration is between 4.0 and 7.4, it has been determined that the formulations won't irritate the eyes. Then, besifloxacin HCl was added to four formulations. These two formulations had a pH of 4.0, were gelated at a temperature between 35 and 34°C, and had pseudoplastic behavior. Additionally, it was shown that both formulations' drug loading capacities exceeded 97%. Four formulas, nevertheless, were made available for six hours. It is believed that increasing a medicine's interaction with Its bioavailability may be increased by the ocular surface. Such medication includes besifloxacin HCl as an example. However, it is believed that conjunctivitis treatment success will be increased by co-administration with an antibiotic derivative. With these four formulations, the medication was released for a longer amount of time than with conventional eye drops, extending its residence time on the eye. The four formulations are therefore viewed as promising besifloxacin HCl drug delivery methods.

AUTHOR CONTRIBUTIONS

Concept: H.K.P.; Design: H.K.P., S.Ü.; Control: H.K.P., S.Ü.; Sources: H.K.P., S.Ü.; Materials: H.K.P., S.Ü.; Data Collection and/or Processing: H.K.P., S.Ü.; Analysis and/or Interpretation: H.K.P., S.Ü.; Literature Review: H.K.P., S.Ü.; Manuscript Writing: H.K.P., S.Ü.; Critical Review: H.K.P., S.Ü.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Lin, H.R., Sung, K.C. (2000). Carbopol/pluronic phase change solutions for ophthalmic drug delivery. Journal of Controlled Release, 69(3), 379-388. [CrossRef]
2. Blondeau, J.M. (2004). Fluoroquinolones: mechanism of action, classification, and development of resistance. Survey of Ophthalmology, 49(2), S73-S78. [CrossRef]
3. Polat, H.K., Pehlivan, S., Özkul, C., Çalamak, S., Öztürk, N., Aytekin, E., Fırat, A., Ulubayram, K., Kocabeyoğlu, S., Irkeç, M., Çalış, S. (2020). Development of besifloxacin HCl loaded nanofibrous ocular inserts for the treatment of bacterial keratitis: *In vitro, ex vivo* and *in vivo* evaluation. International Journal of Pharmaceutics, 585, 119552. [CrossRef]
4. Miller, D. (2013). Pharmacological treatment for infectious corneal ulcers. Expert Opinion on Pharmacotherapy, 14(5), 543-560. [CrossRef]
5. Polat, H.K. (2022). Design of Metformin HCl and Moxifloxacin HCl Loaded Thermosensitive *In Situ* Gel. Journal of Oral Biology and Craniofacial Research, 26(5), 1230-1241. [CrossRef]
6. Lee, V.H. (1990). New directions in the optimization of ocular drug delivery. Journal of Ocular Pharmacology and Therapeutics, 6(2), 157-164. [CrossRef]
7. Polat, H.K. In Situ Gels Triggered by Temperature for Ocular Delivery of Dexamethasone and Dexamethasone/ SBE-β-CD Complex. Journal of Research in Pharmacy, 26(4), 873-883. [CrossRef]
8. Miyazaki, S., Yokouchi, C., Nakamura, T., Hashiguchi, N., Hou, W.M., Takada, M. (1986). Pluronic F-127 gels as a novel vehicle for rectal administration of indomethacin. Chemical and Pharmaceutical Bulletin, 34(4), 1801-1808. [CrossRef]

9. Morishita, M., Barichello, J.M., Takayama, K., Chiba, Y., Tokiwa, S., Nagai, T. (2001). Pluronic® F-127 gels incorporating highly purified unsaturated fatty acids for buccal delivery of insulin. International Journal of Pharmaceutics, 212(2), 289-293. [CrossRef]
10. Dumortier, G., Grossiord, J.L., Agnely, F., Chaumeil, J.C. (2006). A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharmaceutical Research, 23(12), 2709-2728. [CrossRef]
11. Chu, J.S., Amidon, G.L., Weiner, N.D., Goldberg, A.H. (1991). Mixture experimental design in the development of a mucoadhesive gel formulation. Pharmaceutical Research, 8(11), 1401-1407. [CrossRef]
12. Jones, D.S., Woolfson, A.D., Djokic, J., Coulter, W.A. (1996). Development and mechanical characterization of bioadhesive semi-solid, polymeric systems containing tetracycline for the treatment of periodontal diseases. Pharmaceutical Research, 13(11), 1734-1738. [CrossRef]
13. Morsi, N., Ghorab, D., Refai, H., Teba, H. (2016). Ketoroloac tromethamine loaded nanodispersion incorporated into thermosensitive *in situ* gel for prolonged ocular delivery. International Journal of Pharmaceutics, 506(1-2), 57-67. [CrossRef]
14. Gugleva, V., Titeva, S., Ermenlieva, N., Tsibranska, S., Tcholakova, S., Rangelov, S., Momekova, D. (2020). Development and evaluation of doxycycline niosomal thermoresponsive *in situ* gel for ophthalmic delivery. International Journal of Pharmaceutics, 591, 120010[CrossRef]
15. Schmolka, I.R. (1972). Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. Journal of Biomedical Materials Research, 6(6), 571-582. [CrossRef]
16. Ribeiro A, Figueiras A, Santos D, Veiga F. (2008). Preparation and solid-state characterization of inclusion complexes formed between miconazole and methyl-β-cyclodextrin. AAPS PharmsciTech, 9, 1102-1109. [CrossRef]
17. Qi, H., Chen, W., Huang, C., Li, L., Chen, C., Li, W., Wu, C. (2007). Development of a poloxamer analogs/carbopol-based *in situ* gelling and mucoadhesive ophthalmic delivery system for puerarin. International Journal of Pharmaceutics, 337(1-2), 178-187. [CrossRef]
18. Singh, C.L., Singh, A., Kumar, S., Kumar, M., Sharma, P.K., Majumdar, D.K. (2015). Development and validation of different ultraviolet-spectrophotometric methods for the estimation of besifloxacin in different simulated body fluids. Indian Journal of Pharmaceutical Sciences, 77(4), 399. [CrossRef]
19. Karataş, A., Sonakin, O., Kiliçarslan, M., Baykara, T. (2009). Poly (ε-caprolactone) microparticles containing levobunolol HCl prepared by a multiple emulsion (W/O/W) solvent evaporation technique: Effects of some formulation parameters on microparticle characteristics. Journal of Microencapsulation, 26(1), 63-74. [CrossRef]
20. Thakor, S., Vhora, I., Desai, J., Thakkar, S., Thakkar, H. (2012). Physiologically activated phase transition systems for improved ocular retention of ketorolac tromethamine. Journal of Pharmacy Bioallied Sciences, 4(Suppl 1), S6. [CrossRef]
21. Patel, A., Cholkar, K., Agrahari, V., Mitra, A.K. (2013). Ocular drug delivery systems: An overview. World Journal of Pharmacology, 2(2), 47-64. [CrossRef]
22. Pawar, P., Katara, R., Mishra, S., Majumdar, D.K. (2013). Topical ocular delivery of fluoroquinolones. Expert Opinion on Drug Delivery, 10(5), 691-711. [CrossRef]
23. Abd Elhady, S.S., Mortada, N.D., Awad, G.A., Zaki, N.M. (2003). Development of *in situ* gelling and muco adhesive mebeverine hydrochloride solution for rectal administration. Saudi Pharmaceutical Journal, 11, 159-171. [CrossRef]
24. Gilbert, J.C., Richardson, J.L., Davies, M.C., Palin, K.J., Hadgraft, J. (1987). The effect of solutes and polymers on the gelation properties of pluronic F-127 solutions for controlled drug delivery. Journal of Controlled Release, 5(2), 113-118. [CrossRef]
25. Shaikh, R., Singh, T.R.R., Garland, M.J., Woolfson, A.D., Donnelly, R.F. (2011). Mucoadhesive drug delivery systems. Journal of Pharmacy and Bioallied Sciences, 3(1), 89-100. [CrossRef]
26. Dantas, M.G.B., Reis, S.A.G.B., Damasceno, C.M.D., Rolim, L.A., Rolim-Neto, P.J., Carvalho, F.O., Almeida, J.R.G.D.S. (2016). Development and evaluation of stability of a gel formulation containing the monoterpene borneol. The Scientific World Journal, 2016, 7394685. [CrossRef]
27. Fathalla, Z.M., Vangala, A., Longman, M., Khaled, K.A., Hussein, A.K., El-Garhy, O.H., Alany, R.G. (2017). Poloxamer-based thermoresponsive ketorolac tromethamine *in situ* gel preparations: Design, characterisation, toxicity and transcorneal permeation studies. European Journal of Pharmaceutics and Biopharmaceutics, 114, 119-134. [CrossRef]
28. Okur, N., Yozgatli, V., Okur, M.E. (2020). *In vitro–in vivo* evaluation of tetrahydrozoline-loaded ocular *in situ* gels on rabbits for allergic conjunctivitis management. Drug Development Research, 81(6), 716-727. [CrossRef]

29. Kurniawansyah, I.S., Rusdiana, T., Sopyan, I., Ramoko, H., Wahab, H.A., Subarnas, A. (2020). *In situ* ophthalmic gel forming systems of poloxamer 407 and hydroxypropyl methyl cellulose mixtures for sustained ocular delivery of chloramphenicole: Optimization study by factorial design. Heliyon, 6(11), e05365. [CrossRef]
30. Alexandridis, P., Hatton, T.A. (1995). Poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 96(1-2), 1-46. [CrossRef]
31. El-Kamel, A.H. (2002). *In vitro* and i*n vivo* evaluation of Pluronic F127-based ocular delivery system for timolol maleate. International Journal of Pharmaceutics, 241(1), 47-55. [CrossRef]
32. Srivastava, M., Kohli, K., Ali, M. (2016). Formulation development of novel *in situ* nanoemulgel (NEG) of ketoprofen for the treatment of periodontitis. Drug Delivery, 23(1), 154-166. [CrossRef]
33. Öztürk Atar, K. (2022). Development and in-vitro characterization of 1-cysteine loaded alginate beads for oral delivery. Journal of Research In Pharmacy, 26(1), 210-218. [CrossRef]
34. Ashrafi, H., Azadi, A. (2016). Chitosan-based hydrogel nanoparticle amazing behaviors during transmission electron microscopy. International Journal of Biological Macromolecules, 84, 31-34. [CrossRef]
35. Jafari-Aghdam, N., Adibkia, K., Payab, S., Barzegar-Jalali, M., Parvizpur, A., Mohammadi, G., Sabzevari, A. (2016). Methylprednisolone acetate–Eudragit® RS100 electrospuns: Preparation and physicochemical characterization. Artificial cells, Nanomedicine, and Biotechnology, 44(2), 497-503. [CrossRef]