Al-Rafidain J Med Sci. 2024;6(2):56-63
DOI: https://doi.org/10.54133/ajms.v6i2.597

Research Article

Preparation and Evaluation of *in situ* Ophthalmic Gel with a Dual Triggered Mechanism for the Delivery of Gatifloxacin and Betamethasone

Fatima Mustafa Ali*, Athmar Dhahir Al-Shohani

Department of Pharmaceutics, College of Pharmacy, Mustansiriyah University, Baghdad 10011, Iraq

Received: 20 January 2024; Revised: 24 April 2024; Accepted: 27 April 2024

Abstract

Background: Bacterial infections of the eye are treated by administering ophthalmic solutions containing corticosteroids and antibacterial agents. The main challenges faced when used for topical instillation are precorneal fast clearance and multiple applications, particularly with gatifloxacin. ***Objectives***: To develop an ocular gel that utilizes both ion-induced and thermal-sensitive mechanisms to achieve gelation. ***Methods***: We prepared and compared formulations containing different percentages of poloxamer 407 and gellan gum (F1–F24) in terms of gelation temperature, gelling capacity, gelation time, and permeation. We tested the optimum formulation for isotonicity and irritation in rabbits. ***Results***: The formulations' pH varied from 6.7 to 7.3. Formulations that passed the gelation temperature test successfully were F6, F7, F9, and F10. For both drugs (F6, F7, F9, and F10), the drug content percentages ranged from 98.64% to 99.95%. In situ, gels (F6, F7, F9, and F10) showed pseudoplastic shear-thinning rheological behavior, which means that their viscosity decreased as the angular velocity went up. F7, which contains 17% poloxamer and 0.5% gellan gum, had 15 seconds of gelation time at 34ºC and remained in gel form for 270 min. It was isotonic and did not change the size or shape of RBCs when topically applied. The rabbit's eyes did not experience irritation due to the extended release of both drugs. ***Conclusions***: The new *in situ* gel formulation may be a superior alternative to the traditional eye drops of gatifloxacin and betamethasone for ocular infections.

Keywords: Betamethasone, Drug delivery, Gatifloxacin, Gellan gum, *In situ* ophthalmic gel, Poloxamer.

تحضير وتقييم جل العيون الموضعي مع آلية تحفيز مزدوجة لتوصيل غاتيفلوكساسين وبيتاميثازون

الخلاصة

الخلفية: يتم علاج الالتهابات البكتيرية للعين عن طريق إعطاء محاليل العيون التي تحتوي على الكورتيكوستيرويدات والعوامل المضادة للبكتيريا. تتمثل التحديات الرئيسية التي تواجهها عند استخدامها في التقطير الموضعي في التخليص السريع قبل القرنية والتطبيقات المتعددة، خاصة مع الجاتفلوكساسين. **الأهداف**: تطوير هلام العين الذي يستخدم كل من الآليات التي يسببها الأيونات والحساسة للحرارة لتحقيق الهلام. **الطرق**: قمنا بإعداد ومقارنة تركيبات تحتوي على نسب مختلفة من poloxamer 407 وصمغ gellan (F1-F24) من حيث درجة حرارة الهلام، وقدرة التبلور، ووقت الهلام، والتغلغل. اختبرنا التركيبة المثلى لتساوي التوتر والتهيج عند الأرانب. **النتائج**: تراوح الرقم الهيدروجيني للتركيبات من 6.7 إلى 7.3. كانت التركيبات التي اجتازت اختبار درجة حرارة الهلام بنجاح هي F6 و F7 و F9 و F10. بالنسبة لكلا العقارين ، تراوحت نسب محتوى الدواء من 98.64٪ إلى 99.95٪. في الموقع ، أظهرت المواد الهلامية (F6 و F7 و F9 و F10 سلوكا ريولوجيا رقيقا للقص من البلاستيك الكاذب، مما يعني أن لزوجتها انخفضت مع ارتفاع السرعة الزاوية. F7 ، الذي يحتوي على 17٪ بولوكسامير و 0.5٪ صمغ جيلان، كان لديه 15 ثانية من وقت الهلام عند 34 درجة مئوية وظل في شكل هلام لمدة 270 دقيقة. كان متساوي التوتر ولم يغير حجم أو شكل كرات الدم الحمراء عند تطبيقه موضعيا. لم تتعرض عيون الأرنب لتهيج بسبب الإطلاق الممتد لكلا العقارين. **الاستنتاجات**: قد تكون تركيبة الجل الجديدة في الموقع بديلا ممتازا لقطرات العين التقليدية من غاتيفلوكساسين وبيتاميثازون لالتهابات العين.

* ***Corresponding author***: Fatima M. Ali, Department of Pharmaceutics, College of Pharmacy, Mustansiriyah University, Baghdad 10011, Iraq; Email: hokeok00@uomustansiriyah.edu.iq

Article citation: Ali FM PJ, Al-Shohani AD. Preparation and Evaluation of *in situ* Ophthalmic Gel with a Dual Triggered Mechanism for the Delivery of Gatifloxacin and Betamethasone. *Al-Rafidain J Med Sci*. 2024;6(2):56-63. doi: https://doi.org/10.54133/ajms.v6i2.597

INTRODUCTION

Administration of pharmaceutical agents through the ocular route is the main approach to optimizing therapeutic efficacy and minimizing undesirable side effects in managing ocular illnesses [1]. When used in the eyes, traditional liquid formulations aren't very bioavailable because tears are always forming and draining quickly through the nasolacrimal pathway, so they need to be given several times a day [2]. The problem is most apparent while administering treatments for infections, during which most eye drops are often administered at intervals of every 2 hours, particularly throughout the first two days of the treatment regimen [3]. In order to overcome these limitations, researchers developed gels as an effective alternative to transport agents to the ocular site [4]. Using *in situ* formulations allows for the controlled release of medications over a longer period of time, resulting in the prolonged presence of these agents in ocular tissues [5]. One can precisely and easily administer the in situ gel as liquid drops into the eyes. Changes in certain physicochemical parameters, such as pH, temperature, and ionic strength, initiate a sol-gel transition inside the cul-de-sac of the eye upon administration [6]. The formulation of in situ gels often incorporates Poloxamer, a thermoresponsive polymer [8]. This substance demonstrates compatibility with biological systems and can facilitate the transportation of small and large molecules [9]. Carefully manipulating the composition can achieve precise temperature control during the sol-gel transition and subsequent drug release [10,11]. In contrast, gellan gum is an exocellular polysaccharide characterized by its anionic properties. Ophthalmic formulations activated by ion exchange have extensively used it to develop in situ gelling agents [12]. We applied ion-sensitive gellan gum to formulate an in situ gel [13]. Recent studies indicate a preference for using many mechanisms to facilitate gel conversion inside the ocular region. For example, we facilitated the ocular administration of nepafenac using carboxymethyl chitosan and poloxamer, which are pH-induced and thermosensitive materials. Temperatures ranging from 32 to 33°C observed a reduced rate of drug diffusion inside the gel matrix [14]. People often use Gatifloxacin (GTN), a fluoroquinolone antibiotic with broad-spectrum activity, to treat ocular bacterial infections like conjunctivitis [15]. Usually, doctors treat eye bacterial infections by administering ophthalmic solutions, which include corticosteroids and antibacterial agents. Betamethasone sodium phosphate (BSP) is a corticosteroid with demonstrated efficacy in relieving ocular pain, inflammation, and erythema [16]. Using several ocular drops results in suboptimal adherence to therapy and patient pain; therefore, a combination of eye drops is a more favorable approach. In the TobraDex® eye drop formulation [17], tobramycin at a concentration of 0.3% and dexamethasone at a concentration of 0.1% are included. This study aims to develop an ophthalmic *in situ* gel that utilizes both ion-induced and thermal-sensitive mechanisms to achieve gelation.

METHODS

Materials and gel preparation

Gatifloxacin (GTN), betamethasone sodium phosphate (BSP) (Samara drug industry, Iraq), poloxamer 407, gellan gum, sodium chloride (NaCl), sodium bicarbonate (NaHCO3), and calcium chloride (CaCl2) (Sigma-Aldrich, Germany). We purchased BetnesolTM (betamethasone sodium phosphate 0.1%), Gatilox (Gatifloxacin 0.3%) eye drops from a local pharmacy, along with all other solutions. We tested GTN, BSP, gellan gum, poloxamer, and the optimum formula using an FTIR spectrophotometer (Shimadzu) and recorded the results between the wavenumber regions of 500–4000 cm-1 [3]. We produced several in-situ formulations based on the percentages stated in Table 1.

Table 1: Formulation of GTN-BSP ocular *in-situ* gel

Formula	Poloxamer 407	Gellan gum
F1	15	0.1
F2	15	0.3
F3	15	0.5
F4	15	0.7
F5	17	0.1
F6	17	0.3
F7	17	0.5
F8	17	0.7
F9	18	0.1
F10	18	0.3
F11	18	0.5
F12	18	0.7
F13	20	0.1
F14	20	0.3
F15	20	0.5
F16	20	0.7
F17	23	0.1
F18	23	0.3
F19	23	0.5
F20	23	0.7
F21	25	0.1
F22	25	0.3
F23	25	0.5
F24	25	0.7

Values were expressed as percentages. All formulas contained o.3% GTN, 0.1% BSP, and 0.01% Benzalkonium chloride.

Initially, we prepared gellan gum solutions by combining the appropriate amount of gellan gum with deionized water and stirring the mixture overnight [18]. Next, we dispersed a quantity of poloxamer into the gellan gum solution while continuously stirring it for 1 hour. We stored the partially dissolved solutions in a refrigerator at 4oC for approximately 24 hours to achieve a clear solution. Then, the right amounts of GTN (0.3% w/v), BSP (0.1% w/v), and benzalkonium chloride (0.01% w/v) were mixed with a little water. This mixture was then added to the polymer solution while it was being stirred constantly until a uniform solution was made [12].

Clarity and pH measurements

We conducted the clarity assessment by visually inspecting the produced samples. We swirled the samples against a black-and-white background under sufficient illumination. We examined the formulations

to assess their transparency, cloudiness, and the presence of any scattered particles [19]. The pH of the prepared *in situ* gel formulas was detected using a calibrated pH meter [20].

Measurement of gelation temperature

We used the tube tilting method to measure the gelation temperature. We transferred two milliliters of the refrigerated formula to a test tube. We kept the tube in a water bath and gradually increased the water bath's temperature by 2°C every 5 minutes. The gelation was considered to occur when the meniscus of the formula would no longer move upon tilting through a 90 ° angle [20].

Gelling capacity

We assessed the gelling capacity using a tear-simulated fluid (TSF) that contained 0.67 g of sodium chloride, 0.2 g of sodium bicarbonate, 0.008 g of calcium chloride dihydrate, and distilled water q.s. to 100 ml [12]. We placed a single drop of the solution in a test tube containing 2 ml of freshly prepared TSF, and then equilibrated it at 37oC. We visually examined the gel formation and recorded the time for gelation and the duration for the gel to dissolve [21].

Drug content determination

We used the spectrophotometric method to analyze the drug content of the in situ gelling systems. We conducted the analysis by dispersing one gram of the in situ gel sample in 100 ml of phosphate-saline buffer with a pH of 7.4 and subjecting it to sonication for two hours. The resulting mixture was then filtered through a 0.45 µm Millipore filter and analyzed using UV. We measured the absorbance spectrophotometrically at 286 nm and 243 nm for gatifloxacin and betamethasone sodium phosphate, respectively [22,16].

Rheological study

We used a Brookfield viscometer to measure the viscosity. We analyzed the viscosity of each formulation at various speeds (10, 30, 50, and 100 rpm) under non-physiological conditions at 25oC, and under physiological conditions at 37oC with TSF [23].

In vitro drug release and kinetics

We performed the in vitro release of GTN and BSP from in situ gel formulations using a modified method that involved a magnetic stirrer and a dialysis membrane (M. WT 4000 Da). We soaked the dialysis membrane in TSF of pH 7.4 for 24 hours and then opened it from both sides. We tightly sealed one end of the membrane with elastic rubber, leaving the other end open to insert 1 g of the in situ preparation (equivalent to 3 mg of GTN and 1 mg of BSP) [16]. We then securely fastened the open end with a rubber band. A glass rod was used to secure the membrane, which was then submerged in 100 ml of TSF (pH 7.4) at a temperature of 37°C±0.5°C [24] and stirred at a rate of 50 rpm. We collected samples at predetermined intervals (0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 9 hr) and replaced them with 1.0 mL of TSF.

Isotonicity evaluation

We conducted isotonicity testing for the optimum formula and compared it with commercial eye drops. We conducted the testing by mixing a drop from the formula with a few drops of blood, placing it on a slide, and observing the morphology of RBCs under a 45X magnification microscope. We conducted the same procedure on commercial eye drops and compared the results [25].

In vitro trans corneal hydration and permeation

We used the Franz diffusion cell for in vitro and ex vivo corneal permeation studies of GTN and BSP from optimized formulations and controls (commercial eye drops)—BetnesolTM (equivalent to 1 mg of betamethasone sodium phosphate per ml) and Gatilox (equivalent to Gatifloxacin 3 mg per ml, 0.3%). We used a millipore membrane filter (Mwt. 3500 Da) for the in vitro study to mimic the corneal epithelial barrier. At 37±0.5ºC, the receptor compartment was filled with 10 mL of freshly made STF (pH 7.4). We removed the samples from the receptor and used a spectrophotometer to measure absorbance at 286 and 243 nm, respectively, to determine the presence of GTN-BSP. We replaced the membrane for the in vitro permeation study with a goat cornea. We positioned the freshly removed cornea so that its epithelial surface faced the donor and receptor compartments during the fixation of the donor compartment [26]. We used a similar procedure for drug evaluation, as previously described for the in vitro permeation study. Following the permeability study, we weighed the cornea (Wt.), submerged it in one milliliter of methanol, and allowed it to dry overnight at 70oC in a desiccator before weighing it again (Wd) [27]. We calculated the corneal hydration level (HL%) using the following formula:

$$HL\% = (Wt. - Wd)/Wt. \times 100$$

Irritation studies

Six Albino rabbits participated in the Draize irritation experiment. The rabbit eye, specifically the lower cul-de-sac of the conjunctiva, received 0.04 ml of the optimum formula. The eyelids held the eye shut for several seconds after application. We then observe the rabbits' eyes at intervals of 1, 24, 48, and 72 hours after exposure. We evaluated the ocular changes using a scoring system that evaluates any modifications to the eyelids, conjunctiva, cornea, redness, swelling, watering, and iris [11].

Table 2: Characterization results of the prepared formulations

Formula *	pH	Gelation Temp. (ºC)	Gelation Time (sec.)	Gel–sol. Time (min.)	Drug Contents (%)	
					GTN	BSP
F1	7.2±0.87	No gel up to 45	-----	-----	-----	-----
F2	7±0.32	No gel up to 45	-----	-----	-----	-----
F3	6.9±0.34	No gel up to 45	-----	-----	-----	-----
F4	6.7±0.26	No gel up to 45	-----	-----	-----	-----
F5	7.2±0.1	36±0.5	-----	-----	-----	-----
F6	7±0.37	35±1.32	13±2.6	90±3.5	99.23±1.1	99.39±1.01
F7	7.3±0.17	34 ±0.5	15±3	270±5	99.91±0.09	99.95±0.07
F8	6.8±0.3	30 ±1.5	------	-----	-----	-----
F9	7.2±0.72	32±0.5	15±8.66	21±6	98.64±1.8	98.88±1.89
F10	7.1±0.26	30±1.15	10±5	30±9.1	99.45±0.74	99.60±0.61
F11	6.9±0.65	29±1	-----	-----	-----	-----
F12	6.8±0.2	29±0.86	-----	-----	-----	-----
F13	7.2±0.3	27±0.5	-----	-----	-----	-----
F14	7±0.7	28±0.86	-----	-----	-----	-----
F15	6.9±0.41	20±2.7	-----	-----	-----	-----
F16	6.7±0.52	18±0.8	-----	-----	-----	-----
F17	7±0.81	26±1.5	-----	-----	-----	-----
F18	7.2±0.55	24±1.5	-----	-----	-----	-----
F19	7.1±0.65	19±0.5	-----	-----	-----	-----
F20	6.9±0.26	16±2	-----	-----	-----	-----
F21	7.2±0.6	23±1.32	-----	-----	-----	-----
F22	7±0.4	18±0.5	-----	-----	-----	-----
F23	6.9±0.87	18±2	-----	-----	-----	-----
F24	6.9±0.7	15±1	-----	-----	-----	-----

*Only F6, F7, F9, and F10 were tested for gelation time and gel-sol. time. All formulas showed transparent appearance.

Stability study

A better mixture was tested to see what happened to its GT, PH, look, and drug content after being stored at 4±2°C and 25±2°C for one month [28].

Statistical Analysis

We analyzed the collected data using the SPSS version 25 statistical software program for Windows (RRID: SCR 016479). This study used a one-way analysis of variance (ANOVA) to evaluate and compare the data's significance. The predetermined significance threshold was established at $p<0.05$.

RESULTS

We evaluated all prepared formulations for clarity, pH, and gelation temperature, and Table 2 shows the results. In terms of clarity, all formulations were transparent when prepared. The formulations' pH varied from 6.7 to 7.3.

Table 3: Kinetic analysis of betamethasone and gatifloxacin from optimum formula 7

Optimum formula	Zero-order		First-order		Higuchi		Korsmeyer-Peppas		
	K_0	R^2	K_1	R^2	K_H	R^2	K_{KP}	R^2	N
Gatifloxacin	11.145	0.8435	0.206	0.8952	28.212	0.9539	25.654	0.9571	0.555
Betamethasone	11.513	0.8020	0.227	0.9116	29.343	0.9739	29.043	0.9739	0.506

The prepared formulations had different gelation temperatures, as seen in Table 2. Even when the temperature reached 45oC, Formulations F1-F4 did not form any gel, whereas Formulations F11-F24 gelled at a temperature below 30ºC. Only F5-F10 had a gelation temperature between 30ºC and 40ºC. We evaluated the gelation time and gelling capacity of formulations (F6, F7, F9, and F10) that successfully passed the gelation temperature test, and Table 2 displays the results. All formulations had gelation times below 30 seconds and gelling capacities above 20 minutes, with the highest time recorded for F7 (270 min.) compared to other formulations. As shown in Table 2, the percentage of drug content for both drugs in F6, F7, F9, and F10 ranged from 98.64% to 99.95%. Data in Figure 1 (C) displays the viscosity of *in situ* gels (F6, F7, F9, and F10) both with and without TSF. In situ, gels (F6, F7, F9, and F10) showed pseudoplastic flowing or shear-thinning rheological behavior, as shown by a drop in viscosity as angular velocity increased in Figure 1 (A and B).

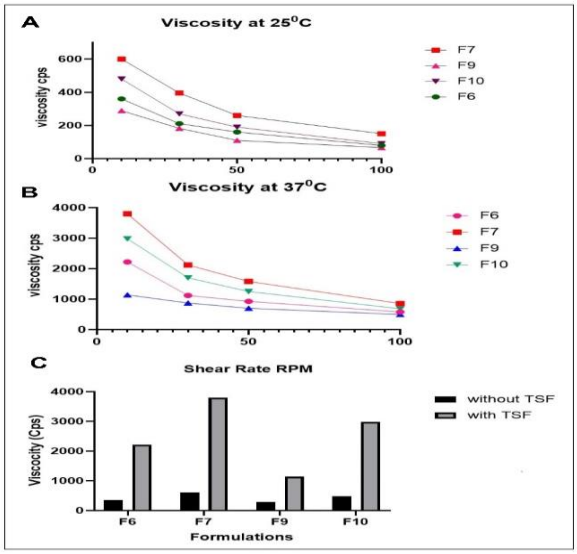

Figure 1: Rheology of in situ gels (F6, F7, F9, F10) (Viscosity *vs*. shear rate of formulations). A) non-physiological at 25ºC (In Sol form); B) under physiological at 37ºC; C) viscosity with and without TSF at 10 rpm.

This happened in both physiological and non-physiological conditions. We conducted drug release from formulations F6, F7, F9, and F10 and compared the results with the release of both drugs from marketed eye drops. Figure 2 illustrates the results of the in vitro release of both BSP and GTN. In the case of eye drops, both drugs released almost all their contents within 1 hour. However, *in situ*, formulations had slower drug release patterns. Regarding BSP, we observed no significant difference ($p>0.5$) in drug release among formulations. However, all formulations had significantly slower drug release ($p<0.5$) compared to eye drops. Similar results were observed for GTN. We chose F7 as the optimal formulation for further studies based on the release results. As shown in Table 3, both drugs follow the Higuchi model of drug release with non-Fickian diffusion.

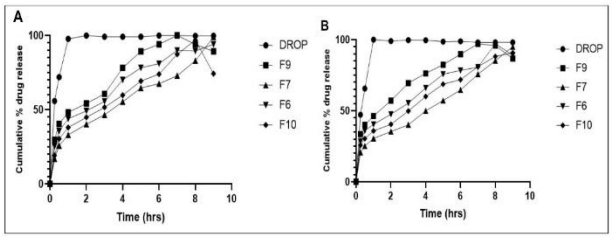

Figure 2: In-vitro release from studied *in-situ* gel formulations in TSF pH 7.4 compared to its eye drop. A) for BSP; B) for GTN.

We tested Formulation F7 for isotonicity, and Figures 3A and B show the results. The application of the in situ gel did not alter the size or shape of the red blood cells, confirming its isotonicity.

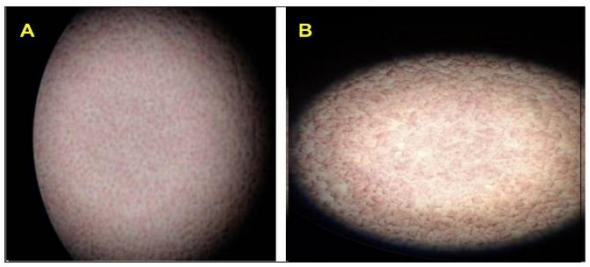

Figure 3: RBCs with A) F7 formula, B) Marketed eye drop.

We also compared F7 with the marketed eye drops. Therefore, we confirmed that the formulation is not harmful to the eye. Figures 4A and B display in vitro permeation studies across the dialysis membrane of optimized F7. After 12 hours of the study, we recorded a cumulative permeation of 93% and 90% for F7 BSP and GTN, respectively. Figures 4A and B display the in vitro drug permeation studies conducted on excised goat corneas using gel formulations and marketed eye drops for BSP and GTN. We used excised goat corneas for permeation studies to mimic real-life conditions, conducting the experiment for 12 hours while considering the cornea's viability. Drug permeation through the cornea from in situ gels ranged between 65 and 63 % for BSP and GTN, respectively, while permeation after using eye drops was 38% and 35 % for BSP and GTN, respectively.

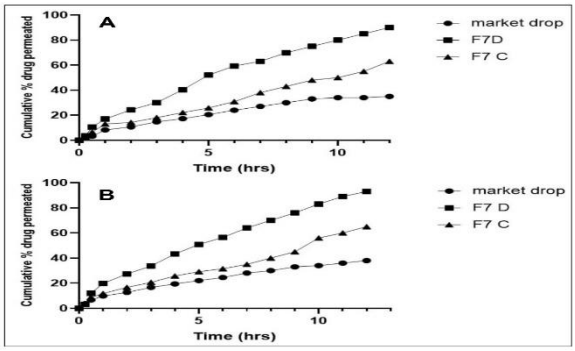

Figure 4: Cumulative permeation of optimized in situ gel formulation (F7) in comparison to marketed eye drop. A) for GTN; B) for BSP from in situ gelling systems through freshly excised goat cornea and dialysis membrane.

We observed lower permeation with goat corneas than with the Millipore membrane filter in 12 hours. We conducted a corneal hydration test on F7 to study that effect. The corneal moisture level was 76.6% after contact, which is within the acceptable range of 76–80% [29]. The outcomes of the ocular irritation studies indicate that all compositions are non-aggravating. As shown in Figure 5, there were no signs of ocular damage or unusual clinical manifestations in the cornea, iris, or conjunctivae.

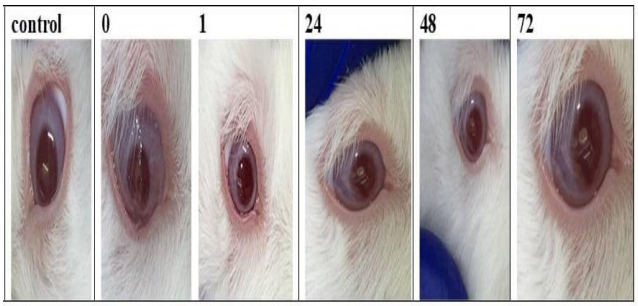

Figure 5: Ocular irritation test on rabbit eye after installation of sterilized F7 eye drop at 0, 1, 2, 24, 48, and 72 hours.

We studied stability for one month at two different temperatures, 25 ºC and 4 ºC, and Table 4 shows the results.

Table 4: Assessment values of pH, drug content, and GT after storage for F7 at 25ºC and 4ºC

Days	pH 25ºC	pH 4ºC	Drug Contents (%)				GT 25ºC	GT 4ºC
			GTN 25ºC	BSP 25ºC	GTN 4ºC	BSP 4ºC		
0	7.30±0.4	7.3±0.45	99.91±0.04	99.95±0.03	99.91±0.04	99.95±0.03	34±3	34±3.4
10	7.28±0.29	7.3±0.3	99.86±0.14	99.90±0.05	99.85±0.14	99.79±0.11	33±1	33±4
20	7.15±0.49	7.25±0.46	99.83±0.15	99.88±0.15	99.72±0.15	99.85±0.05	32±2.6	34±1
30	7.01±0.44	7.28±0.61	99.72±0.19	99.80±0.17	99.63±0.15	99.75±0.19	32±3.6	34±3.4

The optimum formula was physically stable, had no significant changes in any of the parameters assessed during storage, and remained transparent when visually examined. Figure 6 displays GTN's FTIR spectrum, and its characteristic peaks were assigned as follows: 3366 cm^{-1} (O-H group, H-bonded), 2975, 2844 cm^{-1} (C-H group, stretching), 1635 cm^{-1} (C=O group, stretching), 1449 cm^{-1} (C=C group, stretching), 1393, 1365, and 1323 cm^{-1} (C–F group, stretching) [30]. BSP showed IR spectra at 2941 and 2871 cm^{-1} for the stretching of C–H, C−H, 1719 cm^{-1} for the C=O in COO groups, 1665 cm^{-1} for the in-plane deformation vibration of P−O, P−O, and the νCOO, 1602 cm^{-1} for the C−C and νCOO, 1453 and 1393 cm^{-1} for CH, CH2, and δCH3, 1300 cm^{-1} for the coo and δ oh, 1125 cm-1 for the rocking of CH3, ρCH3, and the C-F.

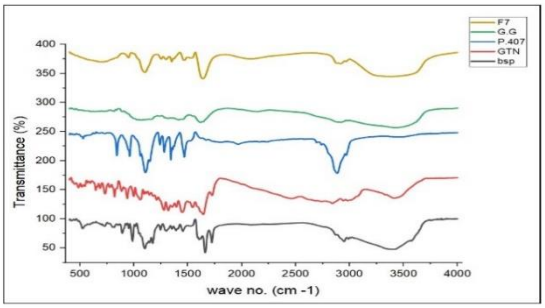

Figure 6: FTIR spectrum for poloxamer 407 (P407), gellan gum (G.G), GTN, BSP, and F7.

The final formulation retained these peaks. The spectrum indicated that the drugs and the gelling agents were compatible with each other [31]. Moreover, the outcomes of the optimal equation (F7) demonstrated no notable shifting in the peaks, indicating the evident compatibility between the two drugs.

DISCUSSION

The present study represents the development and assessment of an *in-situ* gel containing GTN-BSP to treat ocular infections. We developed and evaluated formulations, including poloxamer 407 and gellan gum, two biodegradable and non-toxic polymers, regarding clarity, pH, and gelation temperature. While all formulations exhibited clarity and were within the acceptable pH range for ocular tissues [32], the gelation temperature differed depending on the composition. The absence of gel formation was seen for F1–F4, even at a temperature of 45ºC. One potential rationale for this observation is the somewhat reduced level of poloxamer at a concentration of 15%, despite the concurrent inclusion of gellan gum [33]. A general observation revealed that raising the poloxamer content from 15% to 25% decreased the gelation temperature. The increase in gellan gum content was linked to a concomitant decrease in gelation temperature. We selected Formulas F6, F7, F9, and F10 for further analysis due to their gelation temperature range of 30-35°C. Formula 8,5 exhibited a gelation temperature of 30 and 36ºC, respectively; nevertheless, it was excluded from further testing owing to its notably high viscosity in liquid form during preparation, perhaps attributable to the elevated concentrations of poloxamer and gellan gum [34]. The characteristics of optimal in situ gels are rapid gelation and long-term gel stability. We evaluated the gelation time of in situ gels (F6, F7, F9, and F10). The duration required for the conversion of the solution into a gel is referred to as the gelation time [35]. To prevent medication leakage and dilution during application, it is essential that the gelation period be accelerated [36]. We observed that all formulations had a gelation duration of less than 30 seconds. This characteristic is considered suitable to prevent any potential drug leakage [37]. Gelling capacity refers to the length of time required for the gel to transition from its solid state back to a liquid solution [38]. Formulation F7 had the longest duration of 270 min. in comparison to the other formulations. One potential rationale for this phenomenon is the comparatively elevated concentration of gellan gum in relation to other formulations, thereby enabling the formulation to maintain its gel-like state for a prolonged duration [39]. The observed phenomenon can be attributed to the inherent properties of the polymers, specifically gellan gums, which contain carboxyl and hydroxyl groups [40]. These groups undergo cross-linking reactions when the polymer concentration is increased; consequently, the intermolecular interactions within the polymer matrix are enhanced, forming strong bridges [41]. These bridges contribute to the development of a rigid matrix, thereby influencing the gelling strength. The drug content of the four formulations was found to be within the permitted level, suggesting that there was homogeneity in the distribution of the medication throughout the formulation process. In situ gels (F6, F7, F9, and F10) showed pseudoplastic flowing or shear-thinning rheological behavior, which was shown by a drop in viscosity as angular velocity increased in both physiological and non-physiological settings. The higher viscosity of the gels after mixing with TSF could be explained by the ability of gellan gum to generate gel in work with mono or divalent cations, which are present in TSF and are comparable to the lachrymal fluid [42]. The change in viscosity is proportional to the concentration of gellan gum. The increased viscosity is also affected by the conversion of poloxamer to a gel form at body temperature. As the temperature rises, the poloxamer becomes dehydrated and forms micellar gel. The presence of both mechanisms will provide a synergistic effect and higher viscosity. Researchers conducted a study on medication release, revealing a notable disparity in the release profile compared to commercially available eye drops. In the context of ocular administration, it was observed that almost all of the administered medication was discharged within one hour for both pharmaceutical compounds. Nevertheless, when administered in situ, the formulations exhibited slower drug release patterns. About the BSP, it was found that there was no notable difference in terms of drug release across the various formulations. However, it is

worth noting that all the formulations exhibited much slower drug release when compared to conventional eye drops. Compared to other formulations, F7 demonstrated a comparatively slower release of GTN. Furthermore, all formulations, including F7, showed significantly lower drug release levels compared to eye drops. Many parameters, including the thickness of the gel, the permeability of the gel framework, and the pace of the gel's dissolution, can influence the drug diffusion rate via formulation. The formulation denoted as F7 had the maximum viscosity at physiological temperature, which likely contributed to the comparatively slower rate of drug release from the gel matrix compared to other formulations. The porous nature of the hydrogel allows drugs to be incorporated into the gel matrix, facilitating their sustained release [12]. Based on the release data, we selected the formulation F7 as the optimal choice for further research. All ocular formulations must be isotonic and sterile, so we conducted both tests on F7. The formula was isotonic, and the sterility did not affect the formulation's gelation behavior or pH. As previously mentioned, slow release does not necessarily mean slow permeation, so an in vitro using dialysis membrane and an ex *vivo* using exercised goat corneas permeation study was conducted for F7. Using a dialysis membrane, the cumulative permeation of 93% and 90% after 12 h of the study was recorded for BSP and GTN, respectively. The better permeation that in situ gel showed was probably because of the ability of gellan gum to stick to things and make permeation better. Also, the thick gel that forms blocks drug diffusion [43]. Permeation observed with goat corneas was lower than the permeation observed with the Millipore membrane filter in 12 hours. The cornea, consisting of lipophilic epithelium, hydrophilic stroma, and less lipophilic endothelium, acts as a lipophilic and hydrophilic barrier for corneal penetration, while the dialysis membrane functions as a mechanical barrier [25]. The measurement of corneal HL with the optimized formulation proved the non-damaging effect on the cornea and no irritation was observed when tested on animals. The FTIR results had no evidence of chemical interaction between the two drugs and the stability study for the formulation demonstrated that the formula was stable after one month at both 25ºC and 4ºC storage temperatures.

Conclusion

The *in situ* gel formulation containing GTN and BSP was successfully formulated using poloxamer 407 and gellan gum as gelling agents. The formulation, which contains 17% poloxamer and 0.5 % gellan gum, is converted to gel immediately after application and remains in gel form for 270 minutes. Both drugs had extended gel release compared to eye drops, and the formulation was isotonic with no irritation when tested on rabbit eyes. The findings and evaluations suggest that the formulated GTN and BSP in situ gel can effectively address the limitations of the traditional ocular drug delivery systems.

ACKNOWLEDGMENTS

The authors expressed sincere appreciation to the staff and teaching members of Mustansiriyah University/College of Pharmacy, with whom we have collaborated on this project.

Conflict of interests

No conflict of interests was declared by the authors.

Funding source

The authors did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Gaballa SA, Kompella UB, Elgarhy O, Alqahtani AM, Pierscionek B, Alany RG, et al. Corticosteroids in ophthalmology: drug delivery innovations, pharmacology, clinical applications, and future perspectives. *Drug Deliv Transl Res*. 2021;11(3):866-893. doi: 10.1007/s13346-020-00843-z.
2. Peter M, Panonnummal R. A Review on newer ocular drug delivery systems with an emphasis on glaucoma. *Adv Pharm Bull*. 2021;11(3):399-413. doi: 10.34172/apb.2021.048.
3. Kalam MA, Sultana Y, Samad A, Ali A, Aqil M, Sharma M, et al. Gelrite-based in vitro gelation ophthalmic drug delivery system of gatifloxacin. *J Dispers Sci Technol*. 2008;29(1):89-96. doi: 10.1080/01932690701688482.
4. Shaikh DA, Momin MMJDDL. Formulation and evaluation of ion-triggered in situ gel for effective ocular delivery of ciprofloxacin HCl and olopatadine HCl in combination. *Drug Deliv Lett*. 2024;14(1):49-66. doi: 10.2174/0122103031267809231128111259.
5. Jumelle C, Gholizadeh S, Annabi N, Dana R. Advances and limitations of drug delivery systems formulated as eye drops. *J Control Release*. 2020;321:1-22. doi: 10.1016/j.jconrel.2020.01.057.
6. Tagalpallewar A, Rai P, Polshettiwar S, Manish W, Baheti A. Formulation, optimization and evaluation of ion triggered ophthalmic in situ gel. *J Pharm Res Int*. 2021;33(28A):58-77. doi: 10.9734/jpri/2021/v33i28A31511.
7. Saini R, Saini S, Singh G, Banerjee A. In situ gels-a new trends in ophthalmic drug delivery systems. *Int J Pharm Sci Res*. 2015;6:386-390.
8. Gözcü S, Polat HK, Gültekin Y, Ünal S, Karakuyu NF, Şafak EK, et al. Formulation of hesperidin-loaded in situ gel for ocular drug delivery: a comprehensive study. *J Sci Food Agric*. 2024. doi: 10.1002/jsfa.13407.
9. Gupta B, Mishra V, Gharat S, Momin M, Omri A. Cellulosic polymers for enhancing drug bioavailability in ocular drug delivery systems. *Pharmaceuticals (Basel)*. 2021;14(11):1201. doi: 10.3390/ph14111201.
10. Szalai B, Jójárt-Laczkovich O, Kovács A, Berkó S, Balogh GT, Katona G, Budai-Szűcs M. Design and optimization of in situ gelling mucoadhesive eye drops containing dexamethasone. *Gels*. 2022;8(9):561. doi: 10.3390/gels8090561.
11. Padmasri B, Nagaraju R, Prasanth D. A comprehensive review on in situ gels. *Int J Appl Pharm*. 2020;12(6):24-33. doi: 10.22159/ijap.2020v12i6.38918.
12. Dewan M, Sarkar G, Bhowmik M, Das B, Chattoapadhyay AK, Rana D, Chattopadhyay D. Effect of gellan gum on the thermogelation property and drug release profile of Poloxamer

407 based ophthalmic formulation. *Int J Biol Macromol*. 2017;102:258-265. doi: 10.1016/j.ijbiomac.2017.03.194.
13. Yang H, Ding S, Fan D, Zhu Z, Fan Y, Li J, Wang D. Design and evaluation of a dual-sensitive in situ gel for the controlled release of pranoprofen. *AAPS PharmSciTech*. 2024;25(2):35. doi: 10.1208/s12249-024-02748-3.
14. Yu S, Zhang X, Tan G, Tian L, Liu D, Liu Y, et al. A novel pH-induced thermosensitive hydrogel composed of carboxymethyl chitosan and poloxamer cross-linked by glutaraldehyde for ophthalmic drug delivery. *Carbohydr Polym*. 2017;155:208-217. doi: 10.1016/j.carbpol.2016.08.073.
15. Joshi PH, Youssef AAA, Ghonge M, Varner C, Tripathi S, Dudhipala N, et al. Gatifloxacin loaded nano lipid carriers for the management of bacterial conjunctivitis. *Antibiotics (Basel)*. 2023;12(8):1318. doi: 10.3390/antibiotics12081318.
16. Sabry HS, Al-Shohani ADH, Mahmood SZ. Formulation and evaluation of levofloxacin and betamethasone ophthalmic emulgel. *J Pharm Bioallied Sci*. 2021;13(2):205-211. doi: 10.4103/jpbs.JPBS_338_20.
17. Kolawole OM, Cook MT. In situ gelling drug delivery systems for topical drug delivery. *Eur J Pharm Biopharm*. 2023;184:36-49. doi: 10.1016/j.ejpb.2023.01.007.
18. Huang Y, Yang N, Zhang Y, Hou J, Gao Y, Tian L, et al. The gelling behavior of gellan in the presence of different sodium salts. *Int J Biol Macromol*. 2021;193(Pt A):768-777. doi: 10.1016/j.ijbiomac.2021.10.173.
19. Sun J, Zhou Z. A novel ocular delivery of brinzolamide based on gellan gum: In vitro and in vivo evaluation. *Drug Des Devel Ther*. 2022;16:4109-4110. doi: 10.2147/DDDT.S153405.
20. Al-Bazzaz FY, Al-Kotaji M. Ophthalmic in-situ sustained gel of ciprofloxacin, preparation and evaluation study. *Int J App Pharm*. 2018;10(4):153-161. doi: 10.22159/ijap.2018v10i4.26885.
21. Kalaria VJ, Saisivam S, Alshishani A, Aljariri Alhesan JS, Chakraborty S, Rahamathulla M. Design and evaluation of *in situ* gel eye drops containing nanoparticles of Gemifloxacin Mesylate. *Drug Deliv*. 2023;30(1):2185180. doi: 10.1080/10717544.2023.2185180.
22. Kapoor A, Gupta G. Optimization and characterization of ion activated ocular in-situ gel formulation for bacterial conjunctivitis. *Int J Appl Pharm*. 2020:182-91. doi: 10.22159/ijap.2020v12i4.37925.
23. Amer Z, Mahdi Z, Alhamdany A. Formulation and evaluation of ocular in-situ gelling system containing ciprofloxacin and naproxen sodium. *Res J Pharm Technol*. 2021;14:91-95. doi: 10.5958/0974-360X.2021.00017.2.
24. Mohammadi M, Patel K, Alaie SP, Shmueli RB, Besirli CG, Larson RG, et al. Injectable drug depot engineered to release multiple ophthalmic therapeutic agents with precise time profiles for postoperative treatment following ocular surgery. *Acta Biomater*. 2018;73:90-102. doi: 10.1016/j.actbio.2018.04.037.
25. Arora K, Singh LJJoPNR. Formulation development and characterization of in situ gel containing bimatoprost for the treatment of glaucoma. *J Pharm Negative Results*. 2023:1986-2001. doi: 10.47750/pnr.2023.14.02.247.
26. Majeed A, Khan N. Ocular in situ gel: An overview. *J Drug Deliv Ther*. 2019;9:337-347. doi: 10.22270/jddt.v9i1.2231.
27. Abbas MN, Khan SA, Sadozai SK, Khalil IA, Anter A, Fouly ME, et al. Nanoparticles loaded thermoresponsive in situ gel for ocular antibiotic delivery against bacterial keratitis. *Polymers*. 2022;14(6):1135. doi: 10.3390/polym14061135.
28. Maddiboyina B, Jhawat V, Desu PK, Gandhi S, Nakkala RK, Singh S. Formulation and evaluation of thermosensitive flurbiprofen in situ nano gel for the ocular delivery. *J Biomater Sci Polymer Ed*. 2021;32(12):1584-1597. doi: 10.1080/09205063.2021.1927460.
29. Pramanik A, Sahoo R, Nanda A, Pattnaik K, Mallick S. Swelling kinetics and corneal hydration level of kaolinin-HPMC hydrogel film. *Indian J Pharm Sci*. 2020;82(2):306-314.
30. Arthanari S, Mani G, Jang JH, Choi JO, Cho YH, Lee JH, et al. Preparation and characterization of gatifloxacin-loaded alginate/poly (vinyl alcohol) electrospun nanofibers. *Artif Cells Nanomed Biotechnol*. 2016;44(3):847-852. doi: 10.3109/21691401.2014.986676.
31. Zhang Y, Wu X, Li H, Du N, Song S, Hou W. Preparation and characterization of (betamethasone sodium phosphate intercalated layered double hydroxide)@liposome nanocomposites. *Colloid Surfaces A: Physicochem Engineer Aspects*. 2017;529:824-831. doi: 10.1016/j.colsurfa.2017.06.063.
32. Tassew NG, Laing ST, Aaronson J, de Jong I, Schuetz C, Lorget F. Tolerability assessment of formulation pH in New Zealand white rabbits following intravitreal administration. *Toxicol Pathol*. 2021;49(3):605-609. doi: 10.1177/0192623320969667.
33. Zahir-Jouzdani F, Wolf JD, Atyabi F, Bernkop-Schnürch A. In situ gelling and mucoadhesive polymers: why do they need each other? *Expert Opin Drug Deliv*. 2018;15(10):1007-1019. doi: 10.1080/17425247.2018.1517741.
34. Morsi N, Ibrahim M, Refai H, El Sorogy H. Nanoemulsion-based electrolyte triggered in situ gel for ocular delivery of acetazolamide. *Eur J Pharm Sci*. 2017;104:302-314. doi: 10.1016/j.ejps.2017.04.013.
35. Vigani B, Rossi S, Sandri G, Bonferoni MC, Caramella CM, Ferrari F. Recent advances in the development of in situ gelling drug delivery systems for non-parenteral administration routes. *Pharmaceutics*. 2020;12(9):859. doi: 10.3390/pharmaceutics12090859.
36. Vyas U, Gehalot N, Jain V, Mahajan SC. A Review on in situ gelling system for ophthalmic drug delivery. *Curr Res Pharm Sci*. 2021;11(4):98-106. doi: 10.24092/CRPS.2021.110402.
37. Sha G, Liu J, Jiang Z, Zhu M, Zhu Y, Gu C, et al. In situ gels: The next new frontier in ophthalmic drug delivery system. *Polymer Adv Technol*. 2023;34(8): 2646-2662. doi: 10.1002/pat.6079.
38. Pandya AK, Vora LK, Umeyor C, Surve D, Patel A, Biswas S, et al. Polymeric in situ forming depots for long-acting drug delivery systems. *Adv Drug Deliv Rev*. 2023;200:115003. doi: 10.1016/j.addr.2023.115003.
39. Kurniawansyah IS, Rusdiana T, Sopyan I, Desy Arya IF, Wahab HA, Nurzanah D. Comparative study of in situ gel formulation based on the physico-chemical aspect: Systematic review. *Gels*. 2023;9(8):645. doi: 10.3390/gels9080645.
40. Stachowiak N, Kowalonek J, Kozlowska J, Burkowska-But A. Stability studies, biodegradation tests, and mechanical properties of sodium alginate and gellan gum beads containing surfactant. *Polymers*. 2023;15(11):2568. doi: 10.3390/polym15112568.
41. de Oliveira Cardoso VM, Kiraly VTR, Boni FI, Ferreira NN, Ferreira LMB, Pereira FMV, et al. Rational design of nanocarriers based on gellan gum/retrograded starch exploiting polyelectrolyte complexation and ionic cross-linking processes: A potential technological platform for oral delivery of bevacizumab. *J Drug Deliv Sci Technol*. 2021;66:102765. doi: 10.1016/j.jddst.2021.102765.
42. Nagesh C, Patil M, Chandrashekhara S, Sutar R. A novel in situ gel for sustained ophthalmic delivery of ciprofloxacin hydrochloride and dexamethasone-design and characterization. *Der Pharmacia Lett*. 2012;4(3):821-827.
43. Almeida H, Amaral MH, Lobão P, Lobo JM. In situ gelling systems: a strategy to improve the bioavailability of ophthalmic pharmaceutical formulations. *Drug Discov Today*. 2014;19(4):400-412. doi: 10.1016/j.drudis.2013.10.001.