Development and Evaluation of in situ eye gel for delivery of gatifloxacin and betamethasone sodium phosphate

Fatima Mustafa Ali*, Athmar Dhahir Habeeb Al-Shohani*, Asma Buanz**
Department of pharmaceutics, college of pharmacy, Mustansiriyah university, Baghdad / Iraq
****School of Science, Faculty of Engineering and Science, University of Greenwich, Kent, UK***

Article Info:

Received Oct 2023
Revised Dec 2023
Accepted Feb 2024
Published Jan 2025
Corresponding Author email:
hokeok00@uomustansiriyah.edu.iq
Orcid: https://orcid.org/0009-0009-2913-4591

DOI: https://doi.org/10.32947/ajps.v25i1.1105

Abstract:

Drug delivery to ocular tissues is challenging due to the rapid removal of instilled drugs due to the low resident time in ocular tissues. The study aimed to formulate an ophthalmic in situ gel that delivers two drugs (betamethasone sodium phosphate [BSP] and gatifloxacin [GTN]). The new combination will allow the simultaneous administration and extended release of the two drugs, which could potentially improve resident time in ocular tissues, patient compliance, and treatment adherence.

Formulations containing different gelling agents at different concentrations were prepared to choose the optimum combination regarding physical properties and release. Formulations containing 17% poloxamer 407 and 0.5% gellan gum with different percentages of methylcellulose were prepared and compared regarding gelation temperature, gelling capacity, gelation time, and release and mucoadhesive, permeation study. Increasing the concentration of MC enhanced all the physical properties of the poloxamer-gellan gum gel.

The optimum formula (F3) which contains 0.3% MC had a gelation time of 5 sec. at 31ºC and remained in gel form for 24 hr. Both drugs had extended-release time and increased the viscosity and mucoadhesion force of the preparation. The results indicated an outsized increase in viscosity at 37°C with the addition of MC which provided sustained release of the drug over 10 hours. and the formulation is isotonic to the eye as well no irritation on the rabbit eye was observed when tested on animals.

Conclusion: F3 in situ gel was used to produce a simultaneous and prolonged release of the two drugs. The capacity to administer hydrophilic and hydrophobic medications using a single formulation, eliminating the need for two drops, will increase patient compliance and, consequently, treatment compliance.

Keywords: in situ gel, poloxamer, gellan gum, methylcellulose, betamethasone, gatifloxacin, drug delivery.

تطوير وتقييم جل العين الموضعي لتوصيل جاتيفلوكساسين وبيتاميثازون فوسفات الصوديوم

فاطمة مصطفى علي *, اثمار حبيب*, أسماء بونز**
****قسم الصيدلانيات في كلية الصيدلة الجامعه المستنصرية***
*****كلية العلوم، كلية الهندسة والعلوم، جامعة جرينتش، كينت، المملكة المتحدة***

الخلاصة:
يمثل توصيل الدواء إلى أنسجة العين تحديًا بسبب الإزالة السريعة للأدوية المغروسة بسبب انخفاض وقت الإقامة في أنسجة العين. تهدف الدراسة إلى صياغة هلام عيني موضعي يقدم عقارين) بيتاميثازون فوسفات الصوديوم [BSP] وجاتيفلوكساسين [GTN]).ستسمح التركيبة الجديدة بالإدارة المتزامنة والإفراج الموسع عن العقارين، مما قد يؤدي إلى تحسين وقت الإقامة في أنسجة العين، وامتثال المريض، والالتزام بالعلاج.
تم تحضير تركيبات تحتوي على عوامل تبلور مختلفة بتركيزات مختلفة لاختيار التركيبة المثالية فيما يتعلق بالخصائص الفيزيائية والإطلاق. تم تحضير المستحضرات التي تحتوي على 17% بولوكسامير 407 و 0.5% صمغ جيلان بنسب مختلفة من ميثيل السليلوز ومقارنتها فيما يتعلق بدرجة حرارة التبلور، قدرة التبلور، زمن التبلور، ودراسة التحرر والالتصاق المخاطي. أدت زيادة تركيز MC إلى تعزيز جميع الخواص الفيزيائية لجيل صمغ البولوكسامير جيلان.
الصيغة المثلى (F3) التي تحتوي على MC 0.3 %كان لها وقت تبلور قدره 5 ثواني. عند 31 درجة مئوية ويبقى على شكل هلام لمدة 24 ساعة. كان لكل من العقارين وقت إطلاق ممتد وزاد من قوة اللزوجة والالتصاق المخاطي للتحضير. أشارت النتائج إلى زيادة كبيرة في اللزوجة عند 37 درجة مئوية مع إضافة MC الذي يوفر إطلاقًا مستمرًا للدواء على مدار 10 ساعات. والتركيبة متساوية التوتر بالنسبة للعين، ولم يلاحظ أي تهيج في عين الأرنب عند اختبارها على الحيوانات.
الاستنتاج: تم استخدام هلام F3 في الموقع لإنتاج إطلاق متزامن وطويل الأمد للعقارين. إن القدرة على إعطاء الأدوية المحبة للماء والكارهة للماء باستخدام تركيبة واحدة، مما يلغي الحاجة إلى قطرتين، سيزيد من امتثال المريض، وبالتالي، الامتثال للعلاج.

الكلمات المفتاحية: جل موضعي، بولوكسامير، صمغ الجيلان، ميثيل سيليلوز، بيتاميثازون، جاتيفلوكساسين، توصيل الدواء.

Introduction:

The pharmaceutical market has grown with several medication forms and medical devices in the last two decades, it has played a role in the rise in interest in optical drug administration [1]. However, due to problems in removal, lachrymation, tear flow, metabolism, evaporation, poor absorption and adsorption, limited corneal surface, and low corneal permeability, ensuring adequate delivery remains a major challenge [2]. Most eye drops are used every two hours for curing infections, especially during the first two days of treatment[3]. By enhancing drug viscosity and forming an in-situ gel, increasing eye contact duration can improve this issue and the efficacy of the drugs by lowering systemic absorption, minimizing the dosage's frequency, and raising patients' compliance [4, 5].

Modern alternatives to traditional eye drops include in-situ gel eye drops,[6] which are liquid when it is administered, and immediately gel in the eye's cul-de-sac to create viscoelastic gels[7]. They combine the advantages of gels and liquids such that a solution may be applied to easily give a precise amount, and the gels they create allow for prolonged drug retention and extended-release period of agents in the corneal tissues[8] [9]. Change from solution to gel that happens on the ocular surface is caused by a variety of mechanisms, like temperature-response, ion-activated, and pH-trigger, as well as a combination of more than one mechanism. This ensures effective drug delivery[10] [11].

Poloxamer (P407) as a thermosensitive polymer and gellan gum as ion activated polymer are widely investigated for dual trigger in situ gel formulations. For example, capsazepine with Thermo- response /Pluronic F-127 and pH- trigger /chitosan [12]. For further enhancement of in situ formulations, several techniques embedded in in situ gel (ISG) were used such as nanoparticles, liposomes, nanosuspension, and the addition of viscosity enhancer [13, 14].

Methylcellulose (MC) is extensively used in ocular preparations as a viscosity enhancement. For example Pefloxacin mesylate with Carbopol, Methyl

Cellulose[15]. The addition of MC may further enhance the sustained release of loaded medication.

Gatifloxacin is the 4th-generation antibiotic that is capable of addressing a diverse array of activities, it has superior antibacterial activity. In gram-positive bacteria, it can have a clear antiseptic effect[16]. Antibiotic and corticosteroid eye drops are widely used to treat bacterial eye infections. A corticosteroid called betamethasone sodium phosphate (BSP) aids in reducing discomfort, irritation, and eye redness. its powder appears white and has a molecular weight of 516.4 grams per mole[17]. Combination eye drops are chosen since the use of numerous eye drops reduces patient comfort and treatment compliance. For example, Cipro Dex eye drop eye drop contains (ciprofloxacin 0.3%, and dexamethasone 0.1%)[18].

The objective of the current study is to create an ocular ISG, that undergoes gelling by combining an ion-induced and thermal-sensitive mechanism and examine the impact of the addition of MC in the prepared (ISG) on the physical properties. By prolonging the length of the gatifloxacin and betamethasone residence and increasing the duration of contact on the eye, the formulation provides the potential to reduce the repetition of application. Nevertheless, amalgamating various therapeutic substances and ocular solutions into a single one could amplify patient adherence. Using several ocular drops results in suboptimal adherence to therapy and patient pain; therefore, a combination of eye drops is a more favorable approach. An example is the inclusion of tobramycin at a concentration of 0.3% and dexamethasone at a concentration of 0.1% in the TobraDex® eye drop formulation [19].

MATERIALS AND METHODS:

Materials:

Gatifloxacin (GTN) and betamethasone sodium phosphate (BSP) (a gift from Samara drug industry, Iraq), poloxamer 407, gellan gum (GG), methylcellulose (MC), calcium chloride (CaCl2), bicarbonate of sodium (NaHCO3), and sodium chloride (NaCl) (Sigma-Aldrich. Germany). Betnesol™% (Company GSK, betamethasone sodium phosphate 0.1), Gatilox (company Sun Pharma, Gatifloxacin 0.3%) eye drops (bought from a neighborhood drugstore; all other ingredients, reagents, and chemicals, as well as the solution, were a grade for analysis).

Formulation preparation for ocular ISG:

For the preparation of 50ml of GTN-BSP ocular in-situ gel, all the ingredients shown in Table 1 were accurately weighed, followed by PM solution was prepared using the cold method. by dispersing the polymer in distilled water with continuous stirring for 1 hr. The dispersion was kept in the refrigerator at 4 °C for approximately 24 hrs. to get a completely hydrated dispersion. then the addition of methylcellulose (MC) in different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 w/w %) to a PM dispersion by using the same method. [20]

For gellan gum, solutions were prepared by mixing the required amount in deionized water and stirring overnight at room temperature. The homogenized suspension was stirred for 2 hr. at 70°C and then heated to 90°C for 30 min. till complete hydration [21][3].

On the other hand, the two aqueous solutions of active ingredients (GTN and BSP), along with benzalkonium chloride, were gradually added to the mixture of the previous polymers and stirred for 1hr. after that the solutions were kept refrigerated for 24 hrs. to get a transparent dispersion [22].

Table 1. Formulation of GTN-BSP ocular in-situ gel.

Formula	Poloxamer 407	Gellan gum	MC	GTN	BSP	DW (g)	Benzalkonium chloride
F0	17%	0.5 %	-----	0.3%	0.1%	Up to 10	0.01%
F1	17%	0.5 %	0.1%	0.3%	0.1%	Up to 10	0.01%
F2	17%	0.5 %	0.2%	0.3%	0.1%	Up to 10	0.01%
F3	17%	0.5 %	0.3%	0.3%	0.1%	Up to 10	0.01%
F4	17%	0.5 %	0.4%	0.3%	0.1%	Up to 10	0.01%
F5	17%	0.5 %	0.5%	0.3%	0.1%	Up to 10	0.01%

*Abbreviations: gatifloxacin (GTN), betamethasone sodium phosphate (BSP), methylcellulose (MC), distilled water (DW).
*All the ingredients are expressed in w/w percent.

Characterization of the prepared formula ophthalmic in-situ gel:

Clarity

The prepared samples were visually analyzed to determine the level of clarity. as they were swirled against a background that alternated between black and white, with ample illumination. The formulations were examined to assess their level of transparency, opaqueness, or the presence of any dispersed particles.[23]

pH Measurement

A pH meter was a tool for measuring the pH level of produced (ISG) formulas.[24]

Gelation Temperature (GT) Measurement

For all formulations, the gelation temperatures were measured employing the test tube tilting technique. In this method, the formula was placed in a tube with a capacity of 20 milliliters and submerged in water. The heat of the water in a bath was incrementally raised, resulting in a corresponding alteration of the temperature within the bath. was observed using a digital thermometer. The point at which the solution ceased to flow after slanting the glass tube was noted as the temperature during gelation after tilting the tube 90°C. The accuracy of this procedure was further verified by a viscosity investigation after it was done three times[25].

The tube tilting method was used to measure gelation temperature [12].

Two milliliters of the refrigerated formula were transferred to a test

tube. The tube was maintained in a thermostatically controlled digital

water bath (memmert, Germany) at 4 °C. The temperature of the water

bath was increased gradually in increments of 3 °C at the beginning of

the experiment and then 1 °C increments in the region of sol-gel

transition temperature (25–34 °C) and 0.1 °C when it approaches

gelation. The gelation was considered to occur when the meniscus of the

formula would no longer move upon tilting through a 90 ° angle

The tube tilting method was used to measure gelation temperature [12].

Two milliliters of the refrigerated formula were transferred to a test

tube. The tube was maintained in a thermostatically controlled digital

water bath (memmert, Germany) at 4 °C. The temperature of the water

bath was increased gradually in increments of 3 °C at the beginning of

the experiment and then 1 °C increments in the region of sol-gel

transition temperature (25–34 °C) and 0.1 °C when it approaches

gelation. The gelation was considered to occur when the meniscus of the

formula would no longer move upon tilting through a 90 ° angle

The tube tilting method

was used to measure gelation temperature [12].
Two milliliters of the refrigerated formula are transferred to a st
tube. The tube was maintained in a thermostatically controlled digital
water bath (memmert, Germany) at 4 °C. The temperature of the water
bath was increased gradually in increments of 3 °C at the beginning of
the experiment and then 1 °C increments in the region of sol-gel
transition temperature (25–34 °C) and 0.1 °C when t approaches
gelation. The gelation was considered to occur when the meniscus of the
formula would no longer move upon tilting through a 90 ° angle
The be tilt method was used to measure elation temperature [12].
Two milliliters of the refrigerated formula are transferred to a st
tube. The tube was maintained in a thermostatically controlled digital
water bath (memmert, Germany) at 4 °C. The temperature of the water
bath was increased gradually in increments of 3 °C at the beginning of
the experiment and then 1 °C increments in the region of sol-gel
transition temperature (25–34 °C) and 0.1 °C when t approaches
gelation. The gelation was considered to occur when the meniscus of the
formula would no longer move upon tilting through a 90 ° angle

Gelling capacity

The evaluation of the capacity to form a gel was conducted by utilizing a tear-simulated fluid (TSF) comprising (NaCl) chloride sodium, (NaHCO3) bicarbonate sodium, and (CaCl2) calcium chloride dihydrate (0.67 gram,0.2 gram,0.008 gram) respectively. Distilled water was added to this composition bringing the total volume to 100 milliliters[26]. 0.1 N HCl was used to modify the pH to 7.4[25]. A single drop of the mixture was introduced into a test tube having 2 milliliters of recently prepared TSF, then the test tube was allowed to equilibrate at 37 °C. It was visually seen how a gel formed, and the times it took for it to gel and dissolve were recorded[27].

Determining Drug Content

The spectrophotometric approach was used to analyze the drug content of the produced ISG systems. One gram of the formula was dissolved in Phosphate buffer saline (100 ml), which has a pH of 7.4 and sonicated for two hours to undergo the analysis. The resultant mixture underwent filtration using a 0.45 µm Millipore filter before being subjected to UV analysis. Using a Shimadzu UV/visible double beam spectrophotometer, the absorbance was measured spectrophotometrically at 286 nm and 243 nm [28].

Rheological study

The measurement of viscosity was conducted using the Brookfield Viscometer, specifically the (Brookfield LV model, spindle no. 64). Each formulation's sample was placed within a glass container; then the viscosity of each formulation(ISG) was examined by using various speeds (10, 30, 50, and 100 rpm), and when 1 min had passed between two successful readings, the viscosity of the formulation was measured [29]. The analysis was performed at a temperature of 25°C for non-physiological conditions, and at 37°C with the addition of TSF for physiological conditions[30]. Ophthalmic liquids and gels were subjected to an evaluation of the viscosity of the formula induced by ions., which were formed after the addition of three drops of TSF[31].

Ex vivo Bio adhesive Strength

The bio-adhesive strength was evaluated using a model made from the goat eye [10], bought from a butcher shop and diligently

upheld at a temperature of 37 ºC and a pH level of 7.4 in a solution of phosphate buffer.[32] Using a vial with an eye fitted on one side and a beaker on the other, the modified balancing method was used. The vial's bottom was fitted with the eye. The vials were stored in TSF for 10 minutes at 32-34 °C. The vial containing the eye was then put under a watch glass having a drop of the sample. The first minute of contact between the vial and the gel sample was pushed down. Then, using a pipette, water was gradually poured into the beaker on the other side of the balance [33].

Using a mathematical formula, the quantity of water required to separate the mucous membrane from the gel was evaluated to quantify the mucoadhesive force[34] :

Force of adhesion (N) = (Bioadhesive strength (g) × 9.81) / 1000

Bond strength (Nm^{2-}) = Force of adhesion (N) / Surface area

In vitro drug release study and kinetics

Using a dialysis membrane and a magnetic stirrer, a modified approach was used to release GTN and BSP from the formulations in vitro. The dialysis membrane was opened on both sides and soaked in TSF with a pH of 7.4 for 24 hours. The elastic rubber of the membrane sealed one end firmly while leaving the other open. so that 1 g of the in-situ preparation (corresponding to 3 mg of GTN and 1 mg of BSP) could be inserted. Then, a rubber band was used to firmly tighten the open end of dialysis membrane. The membrane was held in place by the thread on a glass rod. It was followed by submerging in 100 mL of TSF (with a pH level of 7.4) at a temperature of 37°C while it was stirring at a velocity of 50 rpm. At predetermined intervals, 1 mL of the samples were removed, and then more TSF was added. The volume was maintained by the addition of TSF. Using UV light, both medications were examined. The curve fitting technique (using the calibration curve) was used to investigate the release dynamics of the formulations [35].

Isotonicity evaluation

The most optimal formula was subjected to evaluations for isotonicity and its efficacy when compared to commercially available eye drops. To conduct the evaluation, a single drop of the aforementioned formula was mixed with a small quantity of blood, which was subsequently positioned on a slide and observed below a microscope with a magnification of 45X in order to assess the structure of red blood cells (RBCs). This identical process was employed with the commercial ophthalmic drops, and the results were subsequently compared [36].

Antimicrobial study:

The nutrient agar diffusion examination was used to compare the bactericidal effects of GTN from the ideal formula to those of the commercially available GTN eye drops. E. coli, a bacteria Gram-negative bacterium, and Staphylococcus aureus, a Gram-positive bacterium, were used in the experiment, which had a 24-hour growth period. The zone of inhibition was measured after adding a drop of the GTN optimal formula to the agar media to analyze its inhibiting effects and compare them to those of the commercial GTN eye drop[30].

Trans corneal permeation study: in vitro and ex vivo

Franz diffusion cell was employed to conduct an investigation on the permeation of GTN and BSP through the cornea, both in vitro and ex vivo. The study utilized an optimized formulation and a control group consisting of commercial eye drops such as Betnesol™ (containing 1 mg of betamethasone sodium phosphate per mL) and Gatilox (containing 3 mg of gatifloxacin per mL, at a concentration of 0.3%). To simulate the corneal epithelial barrier in the in vitro research, a millipore

layer filter was employed. This layer was positioned between the compartments for donors and receptors, with the test sample being applied to the membrane in the section of donors. Ten milliliters (mL) of TSF were added to the receptor compartment with a pH of 7.4. The donor cell was opened and an aliquot (1 mL) formula was distributed over the Millipore layer filter, followed by the application of a glass coverslip to shut the donor cell aperture. Utilizing a magnetic stir bead, maintain the fluid within the receptor at 37 ± 0.5 °C. The presence of GTN-BSP was determined in samples collected from the receptor by using a UV spectrophotometer to determine absorbance at 286 and 243 nm, respectively. A goat cornea was used in place of the membrane for the ex-vivo permeation research. After the goat was slaughtered, the whole eyes were transferred in cold (4°C) saline solution from the neighboring butcher store to the lab in an hour or less. Then the cornea and two to four millimeters of the surrounding scleral tissue were gently removed, and the area was washed with cold normal saline. During the fixing of the donor compartment, the freshly removed cornea was positioned so that its epithelial layer faced the donor and receptor compartments[37]. To expose the epithelial surface (0.78 cm^2) towards the donor compartment, the cornea was securely attached. The approach employed for drug assessment was the same as the one previously described for the in vitro permeation examination[38].

Cornea hydration

The moisture of the cornea was also investigated in the examination of ex-vivo penetration. After the following permeability research experiment, the cornea was weighed (Wt.), submerged in one milliliter of methanol, dried in a desiccator for one night at 70°C, and then weighed again (Wd.). By measuring the total quantity of water (both free and bound) present by desiccation (gravimetric analysis), the degree of hydration in the cornea was ascertained[10]. The formula below was employed to get the corneal hydration level percentage (HL%):

HL% = (Wt. - Wd)/Wt. x 100

Fourier Transform Infrared Spectroscopy (FTIR) Studies

GTN, BSP, gellan gum, poloxamer, methylcellulose and the optimum formula were tested using FTIR Spectrophotometer (Shimadzu). For all the ingredients, except the formula, the sample to be tested was compressed into a pellet with KBr using a Shimadzu hydraulic press, and the FTIR spectrum was recorded between the wavenumber regions of 500-4000 cm^{-1} on an. The sample was tested in liquid form without modification [39].

Sterilization

The best-prepared formulas were put into glass vials with a capacity of 30 milliliters, sealed with aluminum caps, and fastened with rubber closures. The sealed vials underwent autoclaving for 20 minutes at 121°C and 15 pressure to achieve terminal sterilization[3, 40, 41].

Irritation studies

White Albino rabbits (six)were used in the Draize irritation experiment. The rabbit eye was given 0.04 ml using the ideal formula, more precisely the lower cul-de-sac of the conjunctiva. The eyelids kept the eye closed for a few seconds after application. The eyes of the rabbits are then checked 1-, 24-, 48-, and 72-hours following exposure. A grading system that evaluates any changes to the eyelids, conjunctiva, cornea, redness, edema, watering, and iris was used to analyze the ocular alterations [42].

Stability study

An optimized formulation was noted in the alterations observed in GT, pH, appearance, and percentage of drug content during the course of storage at temperatures of 4 ± 2 °C

as well as 25 ± 2 °C for a duration of 1 month. [43, 44].

Statistical Analysis:
All findings were statistically examined using (one-way ANOVA) to assess and compare the importance of the outcomes, using a significance value of ($P<0.05$).

RESULTS AND DISCUSSION

Formulation preparation for ocular ISG
As seen in Table (2) different formulations containing varying proportions of methylcellulose, along with a consistent amount of poloxamer and gellan gum, were formulated and assessed for their clarity, pH, gelation temperature, gelation time, and gel-sol transition. The evaluation encompassed observations made at both 25 and 37°C in terms of time and appearance.

Clarity and pH
Every formulation was clear when it was developed in terms of clarity. As shown in Table 2, the formulations had pH ranges between 7 and 7.4, which is within the range that ocular tissues may tolerate without becoming irritated [45].

Gelation temperature (GT)
The gelation temperature is a crucial characteristic for making the right formulation decisions. The ideal temperature for best operation is between (30 and 35 °C). As a solution's temperature rises, its viscosity also rises, and the GT shows the temperature at which this shift occurs dramatically[46]. Formulation (F0) without MC gelled at 34°C but the addition of MC slightly reduced the gelation temperature in the other formulas. From Table (2) it is apparent that the GT of PM-G steadily declines when the addition of MC increases. The bond strength between water molecules and poloxamer is reduced by the addition of MC, because MC will form intermolecular hydrogen bonds which means less energy is required to form a gel. To put it another way, a lower temperature is needed for producing a rigid gel. However, because of the MC-poloxamer interaction, a greater MC concentration still led to the creation of a viscous gel. at room temperature. That was observed with F4 and F5 and that is why both formulations were not considered for further investigations. Gelation temperature is low when prepared by using Poloxamer 407 and gellan gum without MC for example (Pilocarpine hydrochloride)[25].

Table 2: Characterization results of the prepared formulations. Only F1-F3 were tested for gelation time and gel-sol. Time.

Formula	pH	Appearance	Gelation temperature (°C)	Gelation time (sec.)	Gel – sol. Time (hr.)	Drug Contents of Formulations	
						GTN %	BSP %
F0	7.3±0.17	Transparent	34 ±0.5	15±3	4.5 ±0.2	99.91±0.09	99.95±0.07
F1	7	Transparent	33 ± 1	10±5	24 ±4	98.26 ±0.4	99.19 ±0.7
F2	7.1	Transparent	32 ± 1.7	5±2	24 ±2	98.98 ±0.7	99.8 ±0.08
F3	7.4	Transparent	31 ±2	5±1	24 ±1	99.34 ±0.9	99.22 ±0.3
F4	7.3	Transparent	29 ±1	-----	-----	-----	-----
F5	7.3	Transparent	26 ±3.2	-----	-----	-----	-----

Gelation time and gelling capacity:

Quick gelation and long-lasting gels are properties of optimum in situ gels. Gelation time was tested for in situ gels (F0- F3). It is the time needed to convert the solution to gel. Gelation time should be fast to avoid drug leakage and dilution during application. The following formulations (F1, F2, and F3) had a gelation time between (15-5) seconds which is optimum for proper use. The addition of MC slightly reduced gelation temperature. Gelling capacity is the period needed for the produced gel to be converted back to solution. While F0 gelling capacity, was only 4:30 hr. the time increased to 24 hr. when MC was added. One reason might be that at lower temperatures when MC is added to this matrix, a rigid gel appears that sticks to the test tube wall more effectively. The reason for the creation of rigid gel is that MC interferes with the PM network, interfering with the hydrogen bonds that form [46].

Determining Drug Content

According to Table 2, the percentage of medication present for both drugs in (F0-F3) ranged from 98.26% to 99.95%, suggesting that the drug was distributed equally and that there was no drug loss during formation.

Rheological study

Measuring the rheological properties of a substance is a significant technique for determining both gelation temperature and gel strength. The strength of the gel directly affects the sustained release property of a drug, measuring rheological properties an indispensable method[47]. the viscosity of a sample greatly influences its residence time, with higher viscosities leading to longer residence times [26]. In both physiological and non-physiological conditions, all formulations exhibited flowing or shear-thinning rheological behavior. This was evident from the decrease in viscosity as the angular velocity increased, as depicted in Figure 1 (A, B). Figure 1C presents the in-situ viscosities of the formulations with and without TSF. The elevated viscosity observed in gels mixed with TSF can be attributed to the gellan gum's ability to generate a gel when in contact with mono- or divalent cations, which are found in TSF and resemble tear fluid [27, 28]. A raised in the amount of MC leads to a corresponding increase in viscosity [8]. Furthermore, the influence of MC during the heating process results in the dissolution of the hydrogen bond between polymer blocks, causing them to aggregate into micelles. As the temperature rises, both the size and quantity of micelles increase, transforming the solution into a more solid gel. Consequently, the introduction of MC further enhances dehydration[48]. Now, adding MC enhances dehydration as earlier. A further observation from Figure 1B is that the mixture comprising PM and MC appears the highest degree of viscosity because a bigger proportion of the OH groups in MC physically cross-linking with the PPO chains of PM, suggesting dehydration, which increases the cross-linking of nearby molecules [46].

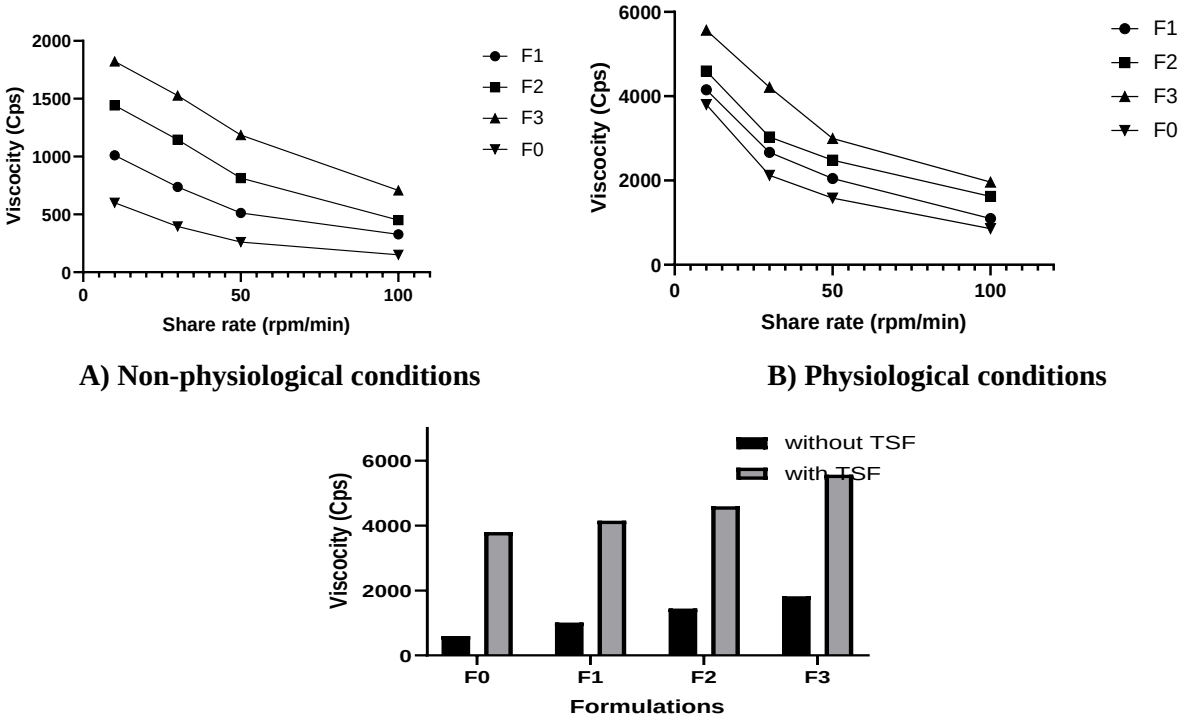


Figure 1. Rheology of in situ gels (F1- F3) (Viscosity vs. shear rate(rpm/min) of formulations) (A) non-physiological at 25 °C (In Sol form), (B) under physiological at 37 °C and (c) viscosity with and without TSF at 10 rpm.

Ex vivo Bio adhesive Strength

In addition to its capabilities as a thickening agent, MC also functions as a bioadhesive polymer[49]. The formulation is more likely to remain inside the eye for an extended period because polymer's propensity to stick to the mucin of the eye. We calculated the gel strength and the sticky force of (F0-F3). The observations in Table 3 clearly show that adding low amounts of MC, like in F1 and F2, slightly increased gel strength and bioadhesion. However, a higher MC content, such as F3, significantly increased gel strength. Bond strength showed a similar pattern.

Table 3: Bioadhesive strength of GTN and BSP in ocular ISG eye drop

Formula No.	Bioadhesive strength (g)	Force of adhesion (N)	Bond strength (Nm-2)
F0	5	0.049	700
F1	5.39	0.052	754.6
F2	6	0.058	828.5
F3	7.6	0.074	1057.1

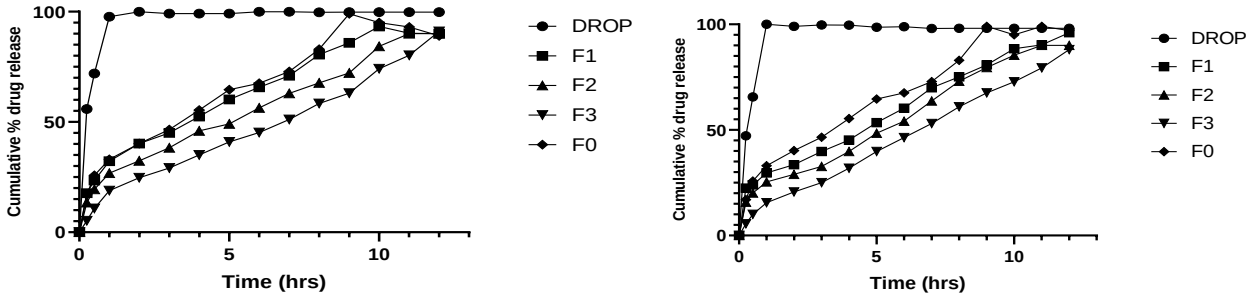
In vitro drug release study and kinetics

Comparing the release of the medicines from formulations F0-F3 with that of the two pharmaceuticals from commercially available eye solutions. The in vitro release's outcomes of both BSP and GTN

were illustrated in Figures 2 A and B. In the case of eye drop, almost all the drug was released within 1 hour for both drugs. However, when in situ formulation F0 was used release of both drugs was significantly reduced and around 90% of the drug was released within 9 hours. When MC was added further slow in drug release was observed and the release order was from fastest to slowest was F1 > F2 > F3, the concentration of MC is crucial for the prolonged release of ocular medications. As MC concentration rose, the amount of medication released gradually decreased as a result. This might be explained by the increasing viscosity and gel strength as MC content rose, which will delay diffusion[50]. Based on the release data, F3 was selected as the best formulation for further research because it has the maximum strength of the gel and thickness of any formulation at physiological condition and has a greater capacity to retain the medication than other formulations. To analyze release patterns from an in-situ gel system, there are three kinetic equations used: Higuchi's equation, first order, and zero order. Since the graphs exhibit the maximum linearity for F3 (r2 > 0.9835, 0.9633 for GTN, 0.9633 for BSP, respectively), it is determined that the Zero order provides the best explanation for the in vitro drug release for both medicines. 'n' ranges between 0.835 and 0.780, which seems to show that non-Fickian diffusion is the only mechanism controlling drug release. The release is within 7 hr. when prepared by using Poloxamer 407 and gellan gum without MC for example (Pilocarpine hydrochloride)[25].

Table (4) Kinetic analysis of BSP and GTN from ideal formula (F3)

Optimum formula F3	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	7.483	0.9835	0.120	0.9680	21.304	0.9239	10.720	0.9940	0.835
Betamethasone	7.515	0.9633	0.122	0.9543	21.490	0.9303	12.130	0.9827	0.780

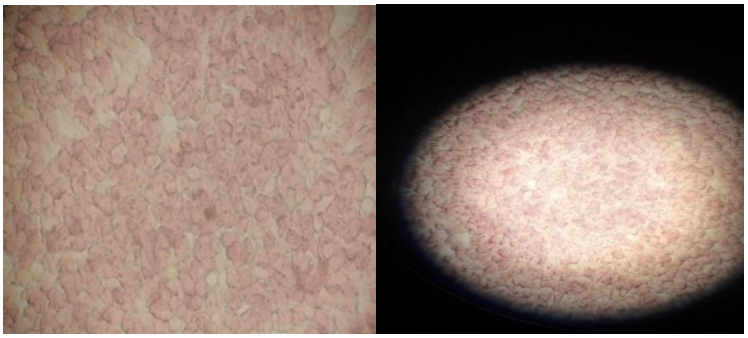


A) BSP B) GTN

Figure 2: In-vitro dissolution from studied in-situ gel product in TSF in pH level 7.4 compared to its eye drop A) for BSP b) GTN.

Isotonicity evaluation:

To prevent irritation, all ocular formulations need to be isotonic. Figures 3A and B show the results of an isotonicity test conducted on Formulation F3. The ISG was isotonic and didn't appear to effect on the size or shape of the red blood cells. Additionally, F3 and commercial eye drops were evaluated. Thus, it was established that the formulation is isotonic to the eye because the polymers are non-electrolytes therefore, they do not exert any or little osmotic pressure on the semipermeable membrane. For example (chloramphenicol, hydroxypropyl methylcellulose (HPMC), Poloxamer 407)[51].



A) F3 B) Market eye drop

Figure 3: RBCs with A) F3; B) Marketed eye drop.

Antimicrobial study:

effectiveness of antibiotics GTN was investigated and contrasted with commercially available GTN eye drops. The findings presented in Table 5 illustrated that GTN retains its capacity to combat microorganisms when incorporated into an ISG formulation in combination with BPS.

Table 5: Zone of inhibition for cultured Staphylococcus aureus and Escherichia coli (E. coli) in both marketed eyes drop and F3.

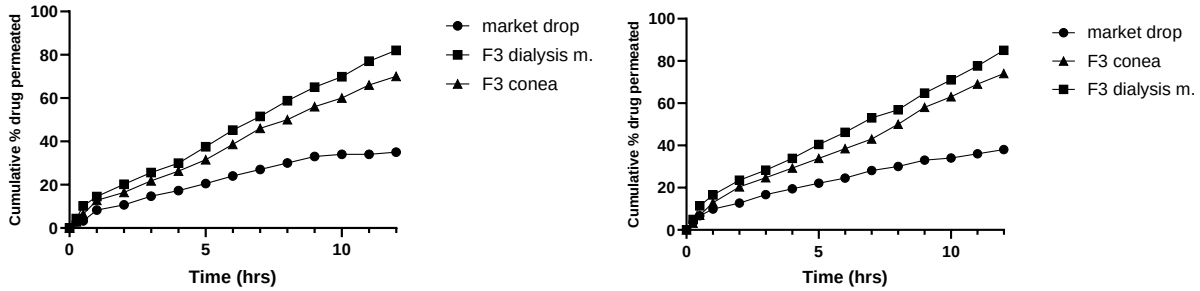
Type of Bacteria	Type of Microorganisms	Zone of inhibition (mm)	
		Marketed antibiotic	F3
Gram +ve	Staphylococcus aureus	27	26
Gram -ve	Escherichia coli (E. coli)	30	20

Trans corneal permeation study: in vitro and ex vivo

Based on previous results, F3 had a slower release so it was chosen as the optimum

Figures 4 A and B show the findings of the in vitro permeation investigation conducted over the improved F3 dialysis membrane. After 12 hours of the trial, F3 BSP and GTN showed cumulative penetrations of 85% and 82%, respectively. Figures 4 A and B show the ex-vivo drug permeation investigations of commercially available GTN-BSP and in situ gel formulations through excised goat corneas. Goat corneas that had been excised were employed in penetration tests to replicate real-world conditions. The

experiment was run for twelve hours, taking into account the viability of the cornea, and the drug permeation from ISG varied from 74 and 70% and 38%, and 35% for eye drops, which was less than the permeation seen in the same period of time using the Millipore membrane filter. Less medication flowed through the corneal membrane than that which was distributed with the dialysis membrane. This might be because the endothelium of the cornea, which is less lipophilic than the epithelium, and the dialysis membrane function as mechanical barriers to prevent corneal penetration [35] The same permeability increase seen by ISG was most likely induced by the bioadhesive of MC.



A- **B-**

Figure 4: Compared to market eye drops, the cumulative permeation of the improved ISG formula (F3). A) GTN, B) BSP from dialysis m. and newly removed goat cornea to in situ gelling systems.

Corneal hydration level

In situ gels are made to hold long-term contact with the cornea to have the most impact, however, this prolonged contact time might harm the corneal endothelium or epithelium. To research this impact, F3 underwent a corneal hydration test. The corneal moisture level was 80% following contact, which is within the recommended range of 76-80%[10].

Fourier Transform Infrared Spectroscopy (FTIR) Studies

As illustrated in Fig. 5, the spectrum of infrared of the GTN drug revealed bands for the tertiary amine C-N (1361 cm-1), florid (1206 cm-1) c=o carboxyl group 1630, unsaturation (3010 and 3076 cm-1), and the carbonyl group of a quinone (1720 cm-1) [52]. For C–H and C–O–C, BSP had infrared spectra at 2943 and 1002 cm-1, respectively. The final formulation kept these peaks in place. For C–H, c=o carboxyl group and C–O , (F3) had infrared spectra at 2990 cm-1,1618 cm-1,1103 cm-1.The spectrum showed that there was compatibility between the medications and the gelling agents[53]. Furthermore, the results of the ideal equation (F3) showed no apparent shifting in the peaks, demonstrating the clear compatibility between both drugs.

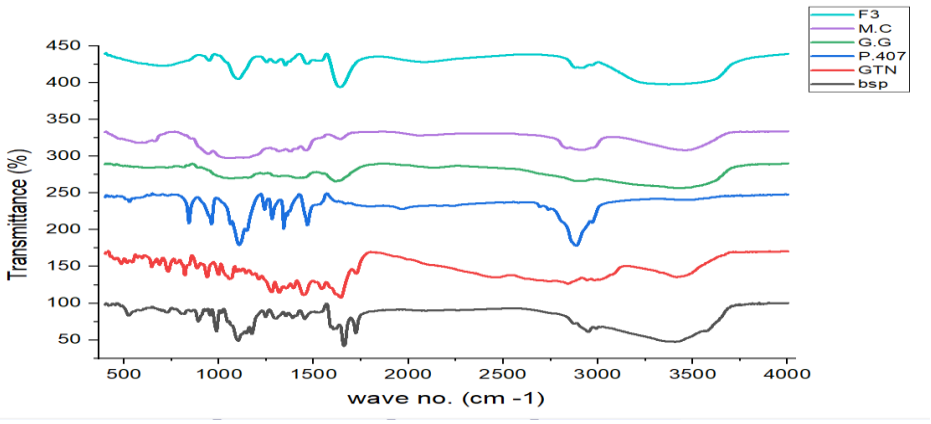


Figure 5: FTIR spectrum for poloxamer 407 (P407), gellan gum (G.G), GTN, BSP, F3.

Sterilization study

Several investigations have examined the stability of poloxamer gels under steam sterilization. there were no changes in the physical and chemical properties, as have the same drug content, pH level, and viscosity. Also, for the FTIR system, there was a little change in intensity but no affected on properties, and for peaks no shift, indicating that the formula is not affected under steam sterilization. As shown in Table 6 and Figure (6,7).

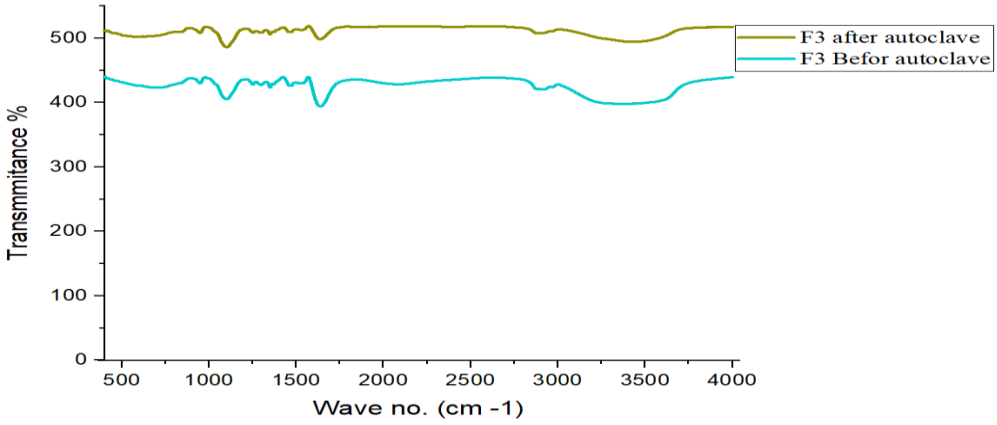


Figure 6. FTIR spectrum for F3 after and before autoclave.

Table 6: The consequences of sterilizing prepared formulations

Formulations	PH		Drug content			
	Before autoclave	After autoclave	GTN		BSP	
			Before autoclave	After autoclave	Before autoclave	After autoclave
F3	7.4±0.1	7.3±0.3	99.34±0.28	99.23±0.14	99.22±0.2	99.10±0.23

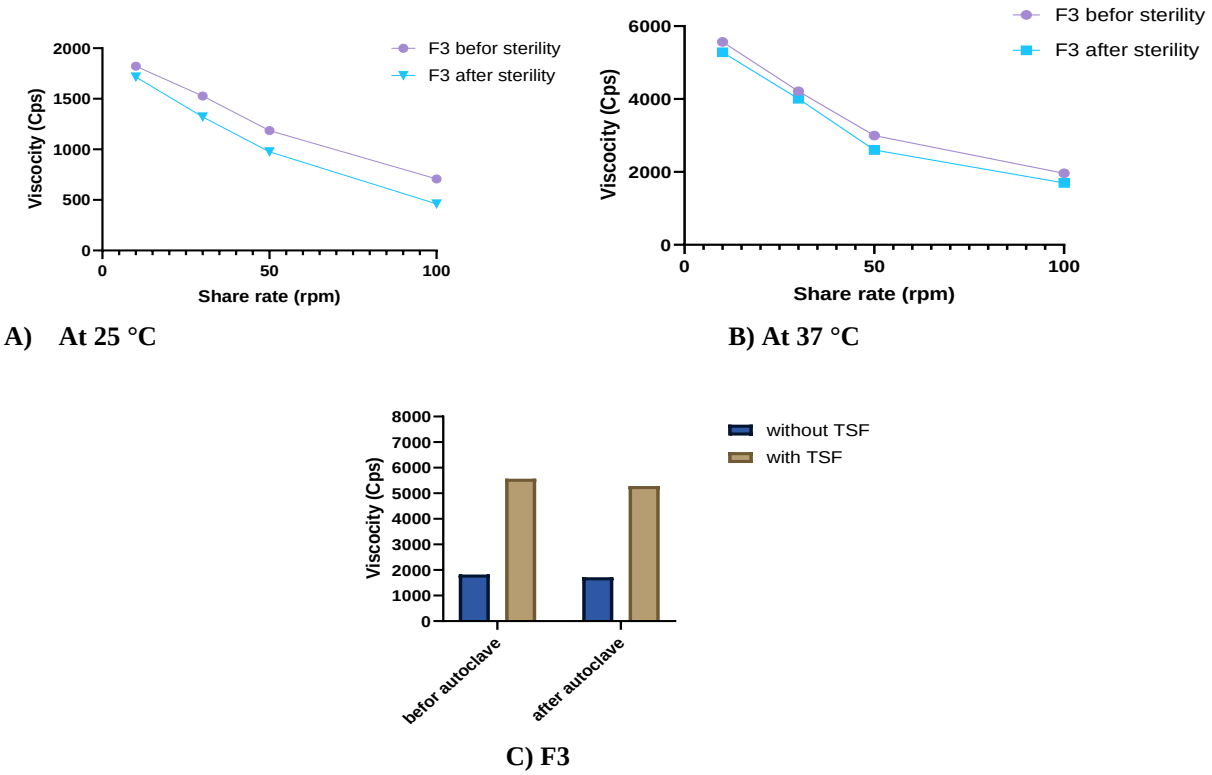


Figure 7. Rheology of in situ gels for (F3) Shows the effect of sterilization on the prepared formulations on (Viscosity vs. shear rate of formulations) (A) non-physiological at 25 °C (In Sol form), (B) under physiological at 37 °C and (c) viscosity with and without TSF at 10 rpm.

Irritation studies

None of the formulations worsen ocular irritation, according to the findings of the research on ocular irritation. The iris, cornea, and conjunctiva didn't exhibit any abnormal clinical symptoms or signs of ocular injury, as Figure 8 illustrates.

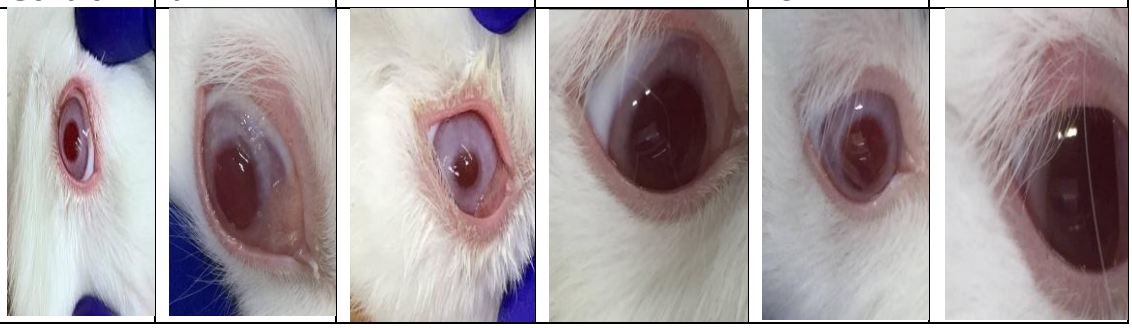
hr.	Control	0	1	24	48	72
F3						

Figure (8): Ocular irritation test on rabbit eye after installation of sterilized F3 eye drop.

Stability study

Table 7 shows the findings of a month-long study on stability conducted at 25 and 4 degrees Celsius. The ideal formula was physically stable, showed no appreciable changes in any of the variables evaluated during storage, and maintained its transparency when visually inspected. [44]

Table 7: Assessment values of pH, drug content, and GT after storage for F3 at 25 °C and 4°C.

Days	pH 25°C	pH 4°C	Drug Contents of Formulations				GT 25°C	GT 4°C
			GTN 25°C	BSP 25°C	GTN 4°C	BSP 4°C		
0	7.4±0.2	7.4±0.1	98.49%	98.89%	98.49%	98.90%	30°C±1	30°C±3.4
10	7.35±0.1	7.3±0.3	98.39%	98.77%	98.35%	99.90%	30°C±2	30°C±4
20	7.26±0.4	7.33±0.36	98.25%	98.65%	98.53%	98.76%	30°C±1.3	30.5°C±1.3
30	7.18±0.1	7.29±0.6	98.20%	98.56%	98.34%	98.70%	30°C±1.8	30.5°C±4.3

CONCLUSION:

The in-situ gel formulation with GTN and BSP was effectively created utilizing poloxamer 407, gellan gum as a gelling agent, and methylcellulose (MC) as a mucoadhesive agent. Formulation, which also contains 0.5% gellan gum, 0.5% poloxamer, and 0.3% methylcellulose, rapidly turns to gel after application and maintains that state for 24 hours. When tested on rabbit eyes, the formulation was isotonic and caused no discomfort. Both drugs released from the gel more slowly than they did from eye drops. According to the results and analyses, Consequently, it may be said that the developed GTN and BSP ISG may effectively solve the disadvantages of the traditional ocular drug delivery methods.

References

1- Alsaidan, O.A., et al., Development of Ciprofloxacin-Loaded Bilosomes In-Situ Gel for Ocular Delivery: Optimization, In-Vitro Characterization, Ex-Vivo Permeation, and Antimicrobial Study. 2022. **8**(11): p. 687.

2- Ranch, K., et al., Development of in situ Ophthalmic gel of Dexamethasone Sodium Phosphate and Chloramphenicol: A Viable Alternative to Conventional Eye Drops. Journal of Applied Pharmaceutical Science, 2017. **7**: p. 101-108.

3- Abul Kalam, M., et al., Gelrite-Based In Vitro Gelation Ophthalmic Drug Delivery System of Gatifloxacin. Journal of Dispersion Science and Technology, 2008. **29**: p. 89-96.

4- Kurniawansyah, I., et al., A REVIEW ON POLOXAMER AND HYDROXY PROPYL METHYL CELLULOSE COMBINATION AS THERMORESPONSIVE POLYMERS IN NOVEL OPHTHALMIC IN SITU GEL FORMULATION AND THEIR CHARACTERIZATION. International Journal of Applied Pharmaceutics, 2021.

5- Gb, P. and E. Narendra, Formulation and evaluation of in situ mucoadhesive ophthalmic hydrogel for sustained delivery of pefloxacin mesylate. International Journal of Pharmacy and Pharmaceutical Sciences, 2015. **7**: p. 345-350.

6- Kumar, L., et al., Insitu gel: A novel system for ocular drug delivery.

International Journal of Pharmaceutical Sciences Review and Research, 2011. **9**: p. 83-91.
7- Wu, Y., et al., Research progress of in-situ gelling ophthalmic drug delivery system. Asian J Pharm Sci, 2019. **14**(1): p. 1-15.
8- Tagalpallewar, A., et al., Formulation, Optimization and Evaluation of Ion Triggered Ophthalmic in Situ Gel. Journal of Pharmaceutical Research International, 2021: p. 58-77.
9- Kadam, A., et al., DESIGN AND EVALUATION OF MODIFIED CHITOSAN BASED IN SITU GEL FOR OCULAR DRUG DELIVERY. International Journal of Pharmacy and Pharmaceutical Sciences, 2017. **9**: p. 87.
10- Morsi, N., et al., Ketoroloac tromethamine loaded nanodispersion incorporated into thermosensitive in situ gel for prolonged ocular delivery. Int J Pharm, 2016. **506**(1-2): p. 57-67.
11- Szalai, B., et al., Design and Optimization of In Situ Gelling Mucoadhesive Eye Drops Containing Dexamethasone. Gels, 2022. **8**(9).
12- Pandey, M., et al., Potential of Stimuli-Responsive In Situ Gel System for Sustained Ocular Drug Delivery: Recent Progress and Contemporary Research. Polymers (Basel), 2021. **13**(8).
13- Kurniawansyah, I.S., et al., Comparative Study of In Situ Gel Formulation Based on the Physico-Chemical Aspect: Systematic Review. 2023. **9**(8): p. 645.
14- Rawat, M. and G.J.J.o.P.N.R. Gnanarajan, Development And In-Vitro Characterization Of Liposomal Embedded In-Situ Ocular Gel Of Dorzolamide Hydrochloride For The Treatment Of Glaucoma. 2023: p. 25-36.
15- Sultana, Y., et al., Evaluation of carbopol-methyl cellulose based sustained-release ocular delivery system for pefloxacin mesylate using rabbit eye model. Pharm Dev Technol, 2006. **11**(3): p. 313-9.
16- Hezhen, T., et al., Effect of gatifloxacin in the treatment of ophthalmological diseases and continuous nursing intervention. Pak J Pharm Sci, 2018. **31**(4(Special)): p. 1707-1712.
17- Sabry, H.S., A.D.H. Al-Shohani, and S.Z. Mahmood, Formulation and Evaluation of Levofloxacin and Betamethasone Ophthalmic Emulgel. J Pharm Bioallied Sci, 2021. **13**(2): p. 205-211.
18- Chan, C.Y., et al., Otologic safety of intratympanic N-acetylcysteine in an animal model. International Journal of Pediatric Otorhinolaryngology, 2023. **173**: p. 111702.
19- Scoper, S.V., et al., Ocular distribution, bactericidal activity and settling characteristics of TobraDex® ST ophthalmic suspension compared with TobraDex® ophthalmic suspension. Advances in Therapy, 2008. **25**(2): p. 77-88.
20- Eaga, C., J. Kandukuri, and V. Allenki, Preparation and Evaluation of In-Situ-Gels for Ocular Drug Delivery. Journal of Pharmacy Research, 2009. **2**.
21- Huang, Y., et al., The gelling behavior of gellan in the presence of different sodium salts. International Journal of Biological Macromolecules, 2021. **193**: p. 768-777.
22- Khule, M. and S. Vyavahare, A Review: in-situ Gel Drug Delivery System. 2021. **9**: p. 2455-6211.
23- Sun, J. and Z. Zhou, A novel ocular delivery of brinzolamide based on gellan gum: in vitro and in vivo evaluation. Drug Des Devel Ther, 2018. **12**: p. 383-389.
24- Albazzaz, F. and M. Alkotaji, Ophthalmic in-situ sustained gel of ciprofloxacin, preparation and evaluation study. International Journal of Applied Pharmaceutics, 2018. **10**: p. 153.
25- Dewan, M., et al., Effect of gellan gum on the thermogelation property and drug

release profile of Poloxamer 407 based ophthalmic formulation. International Journal of Biological Macromolecules, 2017. **102**: p. 258-265.
26- Salmo, H.M., et al., Development and In-Vitro Clinical Study of Tinidazole-Chlorhexidine Gluconate ophthalmic suspension for Treatment of Keratitis. 2007. **4**(1): p. 55-63.
27- Liu, Z., et al., Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin. International Journal of Pharmaceutics, 2006. **315**(1): p. 12-17.
28- Mandal, S., et al., Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. Int J Pharm Investig, 2012. **2**(2): p. 78-82.
29- Ali, M.H.M. and W.K.J.A.M.J.o.P.S. Ali, Preparation and Evaluation of Emulgel as Topical Drug Delivery for Nimesulide by Using Conventional Emulsion. 2019. **19**(4): p. 16-26.
30- Amer, Z., Z. Mahdi, and A. Alhamdany, Formulation and Evaluation of Ocular in-Situ gelling system containing Ciprofloxacin and Naproxen Sodium. Research Journal of Pharmacy and Technology, 2021. **14**: p. 91-95.
31- Khatera, N.A.A., et al., In-situ gelling ophthalmic formulations for sustained release and enhanced ocular delivery of fluconazole. 2016. **11**(2): p. 43-51.
32- Alhaddad, M., Fabrication and characterization of new combination ocular insert for the combined delivery of tinidazole and levofloxacin. 2023.
33- Alkufi, H.k. and H.J. Kassab. Formulation and Evaluation of Sustained Release Sumatriptan Mucoadhesive Intranasal in-Situ Gel. 2019.
34- Wafa, H.G., et al., Ocular films versus film-forming liquid systems for enhanced ocular drug delivery. Drug Deliv Transl Res, 2021. **11**(3): p. 1084-1095.
35- 35. Gadad, A.P., et al., Thermosensitive in situ gel for ocular delivery of lomefloxacin. Indian Journal of Pharmaceutical Education and Research, 2016. **50**: p. S96-S105.
36- Rathore, K.S., Development and in vivo in vitro characterizations of timolol maleate in-situ gels. International Journal of Pharma and Bio Sciences, 2011. **2**: p. 248-263.
37- Pawar, P., et al., Hp-β-CD-voriconazole in situ gelling system for ocular drug delivery: in vitro, stability, and antifungal activities assessment. Biomed Res Int, 2013. **2013**: p. 341218.
38- Abbas, M.N., et al., Nanoparticles Loaded Thermoresponsive In Situ Gel for Ocular Antibiotic Delivery against Bacterial Keratitis. 2022. **14**(6): p. 1135.
39- Kalam, M.A., et al., Gelrite-Based In Vitro Gelation Ophthalmic Drug Delivery System of Gatifloxacin. 2008. **29**: p. 89 - 96.
40- Soliman, K.A., et al., Poloxamer-based in situ gelling thermoresponsive systems for ocular drug delivery applications. Drug Discovery Today, 2019. **24**(8): p. 1575-1586.
41- Deepthi, S. and J. Jose, Novel hydrogel-based ocular drug delivery system for the treatment of conjunctivitis. Int Ophthalmol, 2019. **39**(6): p. 1355-1366.
42- Budumuru, P., R. Nagaraju, and D. Prasanth, A comprehensive review on in situ gels. International Journal of Applied Pharmaceutics, 2020. **12**: p. 24-33.
43- Youssef, A., N. Dudhipala, and S. Majumdar, Ciprofloxacin Loaded Nanostructured Lipid Carriers Incorporated into In-Situ Gels to Improve Management of Bacterial Endophthalmitis. 2020. **12**(6): p. 572.
44- Abraham, S., et al., Sustained ophthalmic delivery of ofloxacin from an ion-activated in situ gelling system. Pak J Pharm Sci, 2009. **22**(2): p. 175-9.

45- Tassew, N.G., et al., Tolerability Assessment of Formulation pH in New Zealand White Rabbits Following Intravitreal Administration. Toxicol Pathol, 2021. **49**(3): p. 605-609.
46- Dewan, M., et al., Effect of methyl cellulose on gelation behavior and drug release from poloxamer based ophthalmic formulations. Int J Biol Macromol, 2015. **72**: p. 706-10.
47- Bain, M.K., et al., Effect of PVA on the gel temperature of MC and release kinetics of KT from MC based ophthalmic formulations. International Journal of Biological Macromolecules, 2012. **50**(3): p. 565-572.
48- El-Kamel, A.H., In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate. International Journal of Pharmaceutics, 2002. **241**(1): p. 47-55.
49- Gupta, B., et al., Cellulosic Polymers for Enhancing Drug Bioavailability in Ocular Drug Delivery Systems. Pharmaceuticals (Basel), 2021. **14**(11).
50- Vodithala, S., et al., Development and evaluation of thermoreversible ocular gels of ketorolac tromethamine. Int J Biopharm, 2010. **1**: p. 39-45.
51- KURNIAWANSYAH, I.S., et al., A review on poloxamer and hydroxy propyl methyl cellulose combination as thermoresponsive polymers in novel ophthalmic in situ gel formulation and their characterization. 2021. **13**: p. 27-31.
52- Arthanari, S., et al., Preparation and characterization of gatifloxacin-loaded alginate/poly (vinyl alcohol) electrospun nanofibers. Artif Cells Nanomed Biotechnol, 2016. **44**(3): p. 847-52.
53- Zhang, Y., et al., Preparation and characterization of (betamethasone sodium phosphate intercalated layered double hydroxide)@liposome nanocomposites. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2017. **529**: p. 824-831.