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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DESIGN AND EVALUATION OF A TEMPERATURE RESPONSIVE *IN SITU* GELLING OPHTHALMIC DRUG DELIVERY SYSTEM CONTAINING BERBERINE HCL

BERBERİN HCL İÇEREN SICAKLIĞA DUYARLI İN SİTU JELLEŞEN OFTALMİK İLAÇ TAŞIYICI SİSTEMİN TASARIMI VE DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *In this study, the efficacy of an in situ gelling formulation developed by combining polymers sensitive to different physiological stimuli for ophthalmic drug delivery systems was investigated. As the active pharmaceutical ingredient, berberine hydrochloride was selected. The primary aim of the study was to design an innovative ophthalmic drug delivery system that offers ease of administration, improves therapeutic efficacy by increasing retention on the ocular surface, and enhances patient compliance by reducing the frequency of dosing.*

Material and Method: *The formulation was prepared using Pluronic F127, chitosan, and κ-carrageenan polymers. Pluronic F127 and chitosan solutions were prepared by the cold method, while the κ-carrageenan solution was prepared at 50°C. The developed system was evaluated in terms of clarity, pH, and gelation temperature. Based on the results obtained, Berberine HCl was added at a concentration of 0.26 mg/ml, in accordance with literature data, to the formulation that exhibited optimal characteristics. The drug release profile was evaluated at 35°C in pH 7.4 isotonic phosphate buffer using the dialysis membrane method. For the stability studies, the formulations were stored for one month at 25 ± 2°C / 60 ± 5% relative humidity and 5 ± 3°C. At the end of the storage period, all analyses were repeated and the drug release profiles were compared using difference (f_1) and similarity (f_2) factors.*

Result and Discussion: *In this study, an in situ gelling ophthalmic drug delivery system containing berberine HCl was developed using a triple polymer system. The results suggest that this system, which may enhance patient compliance, could be a potential alternative to conventional eye drops; however, this needs to be supported by in vivo studies.*

Keywords: *Berberine HCl, in situ gelation, ophthalmic drug delivery system, ophthalmic formulation, polymer based*

ÖZ

Amaç: *Bu çalışmada, oftalmik ilaç taşıyıcı sistemler için farklı fizyolojik uyaranlara duyarlı polimerlerin birleştirilmesiyle geliştirilen in situ jelleşen bir formülasyonun etkinliği araştırılmıştır. Etkin madde olarak berberin hidroklorür seçilmiştir. Çalışmanın temel amacı, uygulama kolaylığı sunan, oküler yüzeyde kalıcılığı artırarak terapötik etkinliği iyileştiren ve doz tekrarını azaltarak hasta uyumunu artıran yenilikçi bir oftalmik ilaç taşıyıcı sistem tasarlamaktı.*

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Gereç ve Yöntem: *Formülasyon, Pluronic F127, kitozan ve κ-karragenan polimerleri kullanılarak hazırlanmıştır. Pluronic F127 ve kitozan çözeltileri soğuk yöntemle, κ-karragenan çözeltisi ise 50°C'de hazırlanmıştır. Geliştirilen sistem berraklık, pH ve jelleşme sıcaklığı açısından değerlendirilmiştir. Elde edilen sonuçlara dayanarak, literatür verilerine uygun şekilde, optimal özellikler gösteren formülasyona 0.26 mg/ml konsantrasyonunda Berberin HCl eklenmiştir. Etken madde salım profili, 35°C'de pH 7.4 izotonik fosfat tamponu içinde diyaliz membran yöntemiyle değerlendirilmiştir. Stabilite çalışmaları kapsamında formülasyonlar 25 ± 2°C / %60 ± 5 bağıl nemde ve 5 ± 3°C'de bir ay boyunca saklanmıştır. Saklama süresi sonunda tüm analizler tekrarlanmış ve etken madde salım profilleri fark (f_1) ve benzerlik (f_2) faktörleri kullanılarak karşılaştırılmıştır.*

Sonuç ve Tartışma: *Bu çalışmada, Berberin HCl içeren, üçlü polimer sistemiyle formüle edilmiş in situ jelleşen bir oftalmik ilaç taşıyıcı sistem geliştirilmiştir. Elde edilen bulgular, hasta uyumunu artırabilecek bu sistemin konvansiyonel göz damlalarına potansiyel bir alternatif olabileceğini göstermektedir; ancak bu durumun in vivo çalışmalarla desteklenmesi gerekmektedir.*

Anahtar Kelimeler: *Berberin HCl, in situ jelleşme, oftalmik formülasyon, oftalmik ilaç taşıyıcı sistem, polimer bazlı*

INTRODUCTION

The primary goal of ocular drugs is to achieve an effective concentration within an adequate time. However, commonly used ophthalmic dosage forms (drops, suspensions, ointments) are not optimal options for treatment. Due to the limited fluid capacity of the eye, which is 7-10 µl, and the corneal barrier, the bioavailability of drugs in drop form is low and requires frequent administration [1,2]. Additionally, drugs entering the systemic circulation through the nasolacrimal drainage can cause side effects, which negatively affect patient compliance. Next generation drug delivery systems are being developed to overcome the limitations of conventional ophthalmic dosage forms. Among these systems, *in situ* gelling drug delivery systems, which are polymer based structures that gel and increase in viscosity upon contact with the ocular surface depending on physiological conditions, hold an important place [3]. This mechanism allows drug delivery systems to remain on the ocular surface for a longer duration, enabling controlled drug release and improving therapeutic efficacy by enhancing bioavailability [4]. Furthermore, their property of being liquid at the time of application and subsequently undergoing gelation can positively influence patient compliance [5]. The localization of *in situ* gelling systems on the ocular surface reduces drug elimination via tears and systemic absorption, thereby minimizing side effects [6]. Gelation mechanisms are primarily dependent on environmental factors such as pH change, temperature increase, or ion activation [7]. The corneal surface temperature is approximately 34°C [8]. Thermosensitive systems frequently used in ocular drug applications exhibit sol gel transition at this temperature. Within this scope, studies have been conducted on polymers such as polyacrylamide derivatives, polyethylene glycol (PEG), polyoxypropylene (PPO) [9], xyloglucan [10], and poloxamer derivatives [11]. The antimicrobial efficacy of ophthalmic *in situ* gelling formulations loaded with azithromycin and ofloxacin, developed using thermosensitive poloxamer derivatives P407 and P188, has been evaluated, showing advantages in bioavailability [12]. In pH-sensitive *in situ* gelling drug delivery systems, polymers that can gel at the corneal surface pH of 7.4 are preferred. For this purpose, carbopol and hydroxypropyl methylcellulose (HPMC) are commonly used. Studies have been conducted on carbopol and HPMC based formulations containing gatifloxacin, which has antimicrobial activity [13]. Moreover, chitosan is a polymer that can gel in response to both temperature and pH changes. Chitosan dissolves at acidic pH and transitions from sol to gel phase as the pH increases [5]. A pH sensitive *in situ* gelling formulation containing timolol, used in glaucoma treatment, has been developed, utilizing carbopol and chitosan polymers in this study [14]. The presence of ions in the tear fluid also influences the gelation process. Therefore, *in situ* gelling drug delivery systems with ion sensitivity are being developed. Ion sensitive systems are known to achieve effective gelation at lower polymer concentrations compared to temperature and pH sensitive systems and have a lower likelihood of causing ocular irritation [15]. Gellan gum, carrageenan, and sodium alginate are considered ion sensitive *in situ* gelling polymers [14,16]. There are studies on gellan gum based ion sensitive formulations loaded with levofloxacin for antimicrobial purposes [17].

Synthetic active substances are generally preferred in the treatment of eye diseases. For instance, artificial tear formulations are commonly used for dry eye syndrome treatment [18], prostaglandin analogs and beta-blockers for glaucoma treatment [19], and antihistamines for conjunctivitis treatment [20]. However, some studies focus on the ophthalmic use of plant derived active substances. Extracts of *Ocimum sanctum and Curcuma longa* are among the plant based components investigated for eye disease treatments due to their antimicrobial and antioxidant properties [21]. Additionally, plant derived components such as allantoin and berberine are known to be included in ophthalmic preparations because of their antimicrobial characteristics [22]. Berberine is a natural bioactive compound belonging to the quaternary isoquinoline alkaloid group. It is abundantly found in the roots and stem bark of species such as *Hydrastis canadensis, Berberis aquifolium, Berberis aristata, and Berberis vulgaris*. Physically, it appears as a yellow, odorless, bitter tasting crystalline powder. While its natural form has very low solubility in water, its solubility and bioavailability improve in its salt form [23]. Berberine is considered a promising therapeutic agent in the treatment of various ocular diseases due to its anti inflammatory and protective properties. Research indicates that berberine exerts therapeutic effects by targeting specific cellular pathways and promoting tissue repair. It has been found effective in treating noninfectious ocular surface inflammations, aiding ocular tissue repair, and increasing the cells responsible for tear production. Due to these properties, it has been reported to reduce inflammation and apoptosis in the treatment of dry eye disease [24]. Its antioxidant effect protects retinal pigment epithelial cells in age related macular degeneration and prevents hydrogen peroxide-induced cell apoptosis [25]. Additionally, its efficacy is being investigated in the treatment of diabetic retinopathy [26] and thyroid-related ophthalmopathy [27].

In this study, the efficacy of combining polymers with different physiological sensitivities in ophthalmic drug delivery systems was investigated. Uniquely, an *in situ* gelling system was developed by using temperature sensitive Pluronic F127 (PF127), pH sensitive chitosan, and ion sensitive kappa-carrageenan polymers together. Berberine hydrochloride, a plant derived active ingredient, was used in the formulation. It was incorporated at a concentration of 0.26 mg/ml, based on the composition of a commercially available preparation abroad [28]. The main aim of the study was to design an innovative drug delivery system that enhances therapeutic efficacy and increases patient compliance by improving the retention of the drug on the ocular surface.

MATERIAL AND METHOD

Materials

The chemical substances used in this study are as follows: Berberine HCl, Pluronic F127, Chitosan, κ-Carrageenan, Acetic acid, Methanol (Sigma-Aldrich); Disodium hydrogen phosphate, Sodium chloride, Ethanol (Merck); Sodium hydroxide (MolyChem), and Potassium dihydrogen phosphate (Deajung).

The main laboratory instruments used in the study include: a UV-Vis spectrophotometer (Thermo Scientific Evolution 201), pH meter (Mettler Toledo Seven Compact S210), viscometer (Brookfield), magnetic stirrer (Daihan Scientific Wisestir SMHS-6), and a temperature-controlled horizontal shaker (GFL 1080).

Preparation of *In Situ* Gel System

The Pluronic F127 and Chitosan solutions were prepared using the cold method [29]. The κ-Carrageenan solution was prepared at a concentration of 0.5% (w/v) in pH 7.4 phosphate buffer at 50 °C. Pluronic F127 and κ-Carrageenan solutions were prepared using pH 7.4 phosphate buffer, while the Chitosan solution was prepared using aqueous acetic acid. All solutions were stirred for 2 hours at 4 °C using a magnetic stirrer and then stored at 5 ± 3 °C for 24 hours. The prepared solutions were subsequently combined to formulate the *in situ* gelling drug delivery system. The composition of the formulation is presented in Table 1. The developed in situ gel systems were evaluated in terms of physicochemical parameters such as clarity, pH, and gelation temperature.

Table 1. Formulation composition of the *in situ* gel system

Formulations	PF 127 (%w/v)	1.5% (w/v) chitosan solution (ml)	0.1% (w/v) chitosan solution (ml)	0.5% (w/v) K-Carrageenan solution (ml)	pH 7.4 buffer solution q.s. to ml
F1	20				10
F2	18				10
F3	17				10
F4	16				10
F5	15				10
F6	18	1			10
F7	18	0.5			10
F8	17	1			10
F9	17	0.5			10
F10	17	0.25			10
F11	16	0.5			10
F12	15	0.5			10
F13	15		0.5		10
F14	15		0.5	2	10

Clarity Analysis

Formulations (2 ml) were visually examined under intense light before and after gelation to assess clarity [30]. The solutions were evaluated for particle content and homogeneity, with the ideal formulations expected to be clear and homogeneous.

pH Measurements

pH measurements were performed using a calibrated pH meter to ensure biocompatibility for ophthalmic use. The formulations aimed to have a pH as close as possible to the tear fluid pH of approximately 7.4.

Gelation Temperature Analysis

The gelation temperature of the formulations was determined by gradually heating the samples in a warm water bath. The solution was heated by the rate of 2 ^{o}C/min with continuous stirring of 100 rpm. The temperature at which the formulation transitioned into a gel was recorded.

Incorporation of Berberine HCl into the Formulation

After determining the optimum formulation, Berberine HCl was added as the active pharmaceutical ingredient. Berberine HCl is available as 0.26 mg/ml ophthalmic solution in commercial formulations, hence same concentration was used in our formulations

The physicochemical stability of the Berberine containing formulation (FA) was reevaluated in terms of clarity, pH, and gelation temperature. In addition, viscosity measurements, *in vitro* drug release and stability studies were also performed. The composition of the Berberine HCl containing formulation is presented in Table 2.

Table 2. Composition of the Berberine HCl Containing Formulation

Formulation	Pluronik F127 (%w/v)	0.1% (w/v) chitosan solution (ml)	0.5% (w/v) K-Carrageenan solution (ml)	Berberine HCl (mg)	pH 7.4 buffer solution q.s. to ml
FA	15	0.5	2	2.6	10

Viscosity Measurement

The viscosity of the formulation containing Berberine HCl was measured using a Brookfield DV-III viscometer. The spindle (96 T-F) runs at 1, 2.5, 5, 10, 20, 50 rpm angular velocity. The viscosity of the *in situ* gelling system was measured at its gelling temperature (32°C). The relationship between viscosity and rotational speed was plotted graphically.

In Vitro Drug Release

The drug release profile was evaluated using the dialysis membrane method [31]. A 100 µl sample of the formulation was loaded into a dialysis membrane. Then dialysis bags were hermetically sealed and dropped into 25 ml of pH 7.4 isotonic phosphate buffer rotating at 100 rpm at 35 °C (ocular surface temperature) At 5, 10, 15, 30, 60, 90, 120, and 180 minutes. Samples were collected, and the concentration of the active substance was determined using a UV spectrophotometer at 348 nm. The release profile was plotted as the cumulative amount of drug released versus time. All experiments were performed in triplicate.

Stability Studies

Prepared formulation (FA) was stored for one month under conditions of 25 ± 2°C / 60 ± 5% relative humidity and 5 ± 3°C. At the end of this period, their physicochemical properties were evaluated and drug release studies were repeated. Formulation stability was assessed by comparing the release profiles using difference factor (f_1) and similarity factor (f_2) analyses. An f_1 value between 0–15 and an f_2 value between 50–100 was considered acceptable limits indicating similarity between release profiles [32].

RESULT AND DISCUSSION

In this study, *in situ* gelling systems containing Berberine HCl were developed and evaluated to enhance drug bioavailability in ophthalmic drug delivery. Considering the limitations of conventional eye drops such as low bioavailability, rapid tear drainage, and frequent dosing requirements the developed system shows potential to overcome these drawbacks**.**

Physical Characteristics of *In Situ* Gelling Berberine HCI Delivery System

The physical suitability of the drug delivery systems developed in this study was evaluated based on gelation temperature and pH values at room temperature. All measurement results obtained are presented in detail in Table 3.

The formulation studies began with the preparation of solutions containing Pluronic F127 at concentrations of 20%, 18%, 17%, 16%, and 15% (F1–F5). All concentrations yielded clear solutions. The gelation temperatures of these formulations were measured as 26℃, 27°C, 28°C, 29°C, and 32°C, respectively (Table 3). The results indicated an inverse relationship between Pluronic F127 concentration and gelation temperature higher polymer content led to lower gelation temperatures.

The gelation of F127, like all Pluronic acids, is significantly affected by temperature. In fact, the gelation behavior of Pluronic acids is characterized by cmc (critical micelle concentration) and cmt (critical micellization temperature). The cmc of F127 decreases significantly with increasing temperature. This show that the gelation temperature also tends to decrease with increasing F127 concentration [33].

Many studies support this finding. This finding is similar to that of Karatas et al. (2015). Pluronic acids are nonionic polyoxyethylene-polyoxypropylene-polyoxyethylene (PEO-PPO-PEO) triblock copolymers. At temperatures below the critical micelle temperature, both PEO and PPO are hydrated. As the temperature increases, the PPO chains dehydrate. This leads to the formation of micelles consisting of a hydrated PEO chain around a dehydrated PPO core. These micelles form a three-dimensional network structure and a rigid gel. Therefore, as the F127 concentration increases, the number of micelles will also increase. Gelation will require less energy and will occur at lower temperatures [29].

Table 3. Evaluation of physical properties of *in situ* gelling berberine HCI delivery systems

Formulations	Gelation Temperature (°C±SD*)	pH (±SD*)
F1	26±2.0	6.90±0.0212
F2	27±1.0	6.90±0.0303
F3	28±1.0	6.90±0.0256
F4	29±1.0	6.90±0.0385
F5	32±2.0	6.90±0.0210
F6	30±1.0	4.50±0.0152
F7	28±2.0	4.76±0.0234
F8	39±1.5	4.95±0.0376
F9	34±1.0	5.12±0.0348
F10	31±0.5	5.38±0.0321
F11	39±1.0	5.27±0.0384
F12	40±1.0	5.22±0.0100
F13	35±0.5	5.51±0.0264
F14	32±1.0	5.90±0.0210

*mean ± SD, n= 3

In our F1-F5 formulations, gelation temperatures increased with decreasing PF127 concentration. Therefore, in selecting the ideal formula, the formula containing the PF127 concentration whose gelation temperature is closest to the corneal temperature was preferred. The pH of all formulations was found to be 6.9, suggesting that the concentration of Pluronic F127 did not significantly influence pH.

Based on the gelation temperature and pH data, chitosan was selected as the second polymer to be incorporated into the formulations. As reported in the literature, chitosan is only soluble in acidic media; therefore, a 1.5% (w/v) chitosan solution was prepared in 2% aqueous acetic acid. This solution was added to the 18% Pluronic F127 formulation in volumes of 1 ml and 0.5 ml to prepare formulations F6 and F7. The gelation temperatures of these formulations were recorded as 30°C and 28°C, with pH values of 4.5 and 4.76, respectively (Table 3).

Similarly, the same chitosan solution was added to the 17% Pluronic F127 base in volumes of 1 ml, 0.5 ml, and 0.25 ml, yielding formulations F8, F9, and F10. Gelation temperatures were found to be 39°C, 34°C, and 31°C, respectively, while the pH values were 4.95, 5.12, and 5.38. Formulations F11 and F12 were obtained by adding 0.5 ml of the chitosan solution to Pluronic F127 at 16% and 15%, respectively. Their gelation temperatures were 39°C and 40°C, with pH values of 5.27 and 5.22. These findings revealed that the addition of chitosan increased gelation temperature and decreased pH due to the acetic acid content.

To optimize pH values, the concentrations of chitosan and acetic acid were reduced. A 0.1% chitosan solution was prepared in 0.5% aqueous acetic acid, and the resulting formulation (F13) gelled at 35°C at pH 5.5.

Chitosan is a biodegradable polymer with excellent skin compatibility. The positively charged amine groups in its chemical structure interact with the negative charge of the eye mucosa, providing it with excellent mucoadhesive properties. Thus, chitosan solutions successfully prolong contact time with the ocular surface. Combining formulations of this polymer with poloxamer offers considerable promise for ocular application due to its increased mechanical strength and extended contact time in the eye [34]. Chitosan gelation is affected by changes in pH and temperature. Chitosan ionizes due to electrostatic forces. The gelation mechanism relies on changes in its critical solution temperature [35].

Following this, κ-carrageenan was selected as the third polymer. A 0.5% (w/v) κ-carrageenan solution was prepared by dissolving the polymer at 50°C. Since the solution solidified at room temperature, it was added dropwise to the formulation while still warm, yielding formulation F14. Gelation temperature of this formulation was found to be 32 °C at pH 5.9. And it appeared clear upon visual inspection.

κ-carrageenan is a sulfated marine polymer. It gels in the presence of ions, producing a strong mucoadhesive effect. A hot aqueous solution of κ-carrageenan has the ability to form thermoreversible gels upon cooling. This is due to the formation of the double-helical structure found in carrageenans. At temperatures above the gel's melting point, the carrageenan polymers are randomly distributed. Upon cooling, they coalesce to form a three-dimensional gel. The presence of chain links and the type, amount, and location of ester and sulfate groups influence carrageenan gelation. To obtain a gel, the solution must contain potassium salts [16].

For the addition of the active pharmaceutical ingredient, the drug delivery system must be capable of gelling at ocular surface temperature. Additionally, the formulation must meet ophthalmic application requirements in terms of pH. The pH of tear fluid is approximately 7.4 and ophthalmic preparations are generally formulated at a pH below 7 [36]. Due to its strong buffering capacity, tear fluid can tolerate formulations with pH values between 3.5 and 10 [37]. F14 formulation, which contains all three polymers, enabled the formation of a stronger gel structure. The formulation F13, with a gelation temperature of 32°C and a pH of 5.9, was found to be suitable for preparation by adding Berberine HCl. And it complies with the criteria for ophthalmic application. The sol to gel transition of the drug delivery system at its gelation temperature is illustrated in Figure 1.

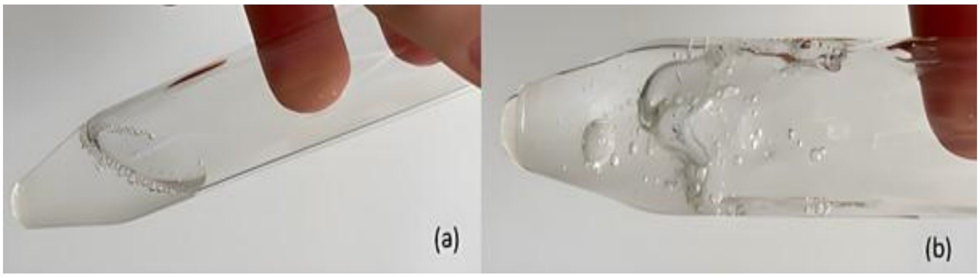

Figure 1. Gelation of the prepared *in situ* gelling system upon temperature increase (a) The formulation exhibiting free flow before the temperature increase, (b) The formulation forming a gel after the temperature increase

Evaluation of Berberine HCl Containing Formulations

Formulations with added Berberine HCl were visually examined to assess their clarity. The evaluations showed that the formulations containing Berberine HCl maintained their clarity. The gelation temperature was measured as 30 ± 1°C and the pH value as 6.5 ± 0.01. These values are considered suitable for ophthalmic preparations. The formulation with the active ingredient was also evaluated in terms of viscosity, *in vitro* drug release, and stability.

Viscosity measurements were performed at the gelation temperature, and the values were recorded. When examining the relationship between shear rate and viscosity, the formulation was found to exhibit pseudoplastic flow behavior. This property indicates that the formulation flows easily during application, allowing for comfortable administration, and subsequently increases in viscosity, prolonging its residence time on the ocular surface [38]. This enhances the efficacy of ophthalmic preparations and supports patient comfort [39]. The rheogram showing viscosity values versus shear rate is presented in Figure 2.

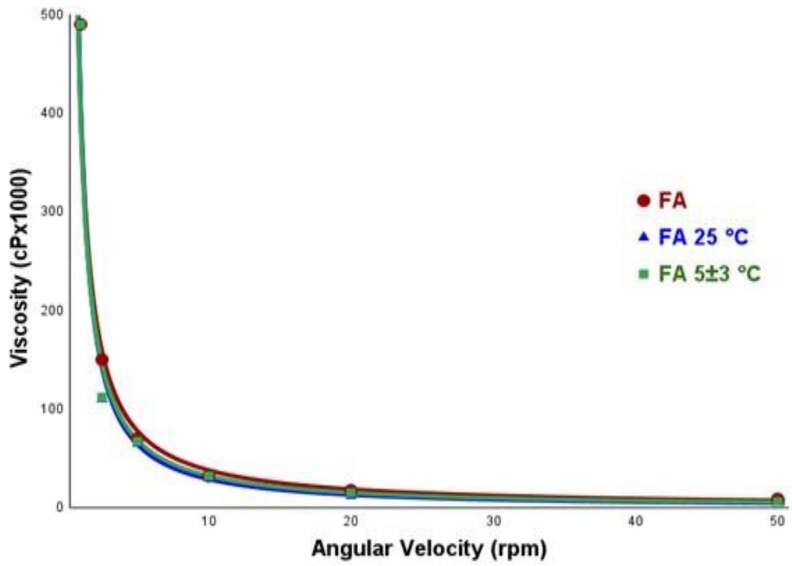

Figure 2. Rheogram of the viscosity measurements of the FA formulation containing Berberine HCl at the beginning (FA) and storage conditions (25 ºC and 5 ± 3°C)

The release profile of Berberine HCl from the prepared formulation was examined. The amount of Berberine HCl released at specific time intervals was measured. It was determined that 17% of the active ingredient was released within the first hour, increasing to 22% by the second hour and 24% by the third hour. Berberine HCI release profile is presented in Figure 3.

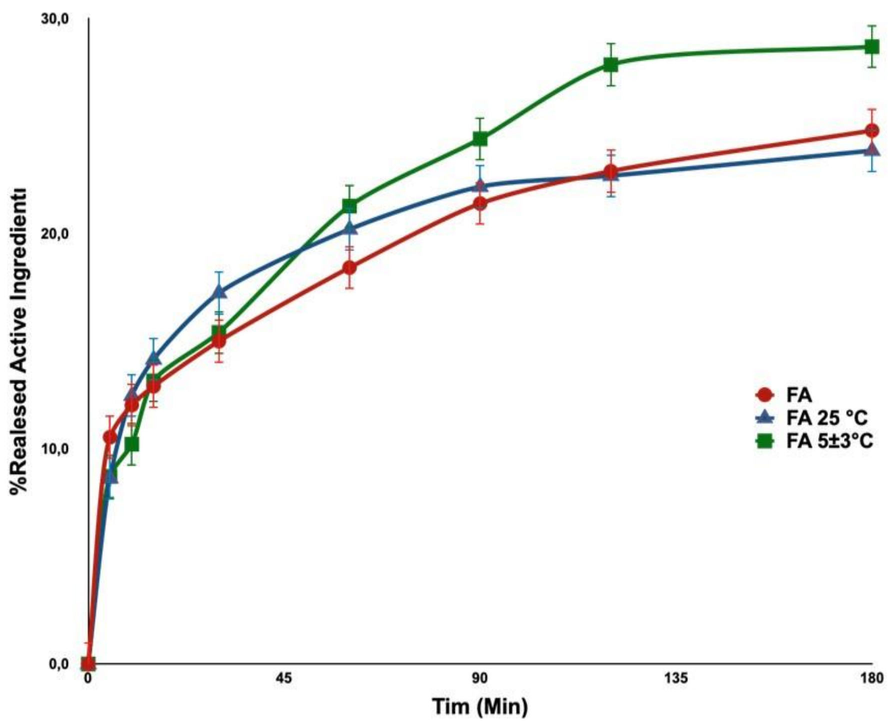

Figure 3. *In vitro* drug release profiles of formulations containing berberine HCl at initial and stability conditions (25 ºC and 5 ± 3°C) (n=8±SE)

The stability of the formulations containing Berberine HCl was assessed over one month under room temperature and refrigerated storage conditions. The evaluation revealed that the formulation maintained its clarity, gelation temperature, and pH value throughout the storage period. Additionally, viscosity changes during the stability study were examined, and it was observed that the initial viscosity values were preserved (Figure 2). When comparing the drug release profiles, the difference (f1) and similarity (f2) factors were calculated as 0.0696 and 84.99, respectively, for the formulation stored at 25°C ± 2°C / 60% ± 5 relative humidity, and 0.1533 and 85.16 for the formulation stored at 5 ± 3°C. These results indicated no significant changes in the release profile during the stability period (Figure 3).

The findings highlight the potential of *in situ* gel forming systems containing Berberine HCl in ophthalmic preparations. The developed formulation has an appropriate gelation temperature and pH for the ocular surface, demonstrating good retention on the eye and improved patient compliance. Its favorable pH, viscosity, and gelation temperature properties contribute to improved compatibility with ocular physiology. By extending the residence time on the ocular surface, the formulation may reduce the need for frequent dosing. The gelation temperature within the 30 ± 1°C range supports controlled gel formation upon contact with tear fluid. Moreover, the preservation of the system's physical properties over one month is a notable outcome in terms of stability.

Although the *in vitro* evaluations provided promising results, further *in vivo* studies are essential. Long residence drug delivery systems require detailed investigation for biocompatibility and potential irritation. Future research could explore how different polymer combinations influence the *in situ* gelation mechanism, contributing to the development of more stable and long acting drug delivery systems. *In vitro* mucoadhesive tests should be conducted to determine whether the mucoadhesive properties of chitosan are maintained after incorporation into poloxamer gels that exhibit high mechanical strength at eye temperature. These tests will also assess whether there is a relationship between chitosan concentration and mucoadhesive properties. In addition, clinical studies may help better understand the efficacy of *in situ* gelling systems.

In conclusion**,** this study demonstrates that *in situ* gel forming ophthalmic drug delivery systems containing Berberine HCl may offer advantages over conventional eye drops and serve as an innovative alternative in ocular drug delivery technologies. The formulation developed in this study includes three different polymers with distinct gelation mechanisms. The combination of these polymers enabled the formation of a strong gel structure. This multi polymer approach enhanced the physicochemical properties of the formulation, standing out as an innovative strategy in the design of drug release systems.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

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

ETHICS COMMITTEE APPROVAL

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

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