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ORIGINAL ARTICLE

Timolol Maleate *in Situ* Ophthalmic Mucoadhesive-Thermosensitive Gel: Development and Characterization

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ABSTRACT

Objectives: The aim of this study was to prepare a sustained-delivery mucoadhesive-thermosensitive formulation containing poloxamer 338 (P338), poloxamer 188 (P188), and mucoadhesive agents, such as chitosan (CHT) and carboxymethylcellulose (CMC), to increase the ophthalmic bioavailability of timolol maleate (TM).

Materials and Methods: Gels were prepared by mixing different amounts of P338, P188, and a mucoadhesive agent in cold isotonic water using a magnetic stirrer. The sol-gel gelation time of the gels was determined using the test tube inversion method. Viscosity measurements and analysis of the mechanical properties of the gel formulations were performed. *In vitro* release using dialysis membranes and *ex vivo* permeation studies using fresh-warmed cow eyes were performed.

Results: The gelation times of formulations containing 20:2.5 (P338:P188) and 0.1% CMC and formulations containing 20:2.5 (P338:P188) and 0.1% CHT were 35 s and 26.67 s, respectively. An optimally selected CHT mucoadhesive-thermosensitive *in situ* gelling system can successfully control the release of moderately hydrophilic drugs, such as TM. In the viscosity study, both formulations showed Newtonian fluid, and the CHT gel's viscosity was found to be higher. The CHT gel showed better mechanical properties than the CMC gel. The amount of TM penetrating the cow cornea after 24 hours was 73.38%, 71.80%, 67.25%, and 60.55% from the CHT gel, CMC gel, TM solution, and commercial preparation, respectively.

Conclusion: The improved mucoadhesive-thermosensitive *in situ* gelling system successfully controlled the release of TM. The significantly lower drainage of TM into the circulation compared with eye drops is an advantage in treating glaucoma, and the use of mucoadhesive agents increases drug penetration.

Keywords: Timolol maleate, mucoadhesive-thermosensitive, poloxamer, chitosan, carboxymethyl cellulose, ophthalmic gel

INTRODUCTION

Glaucoma is an eye disease that occurs when the balance between the amount of intraocular fluid produced and the amount drained out is disrupted, and it can cause irreversible blindness if left undiagnosed and untreated.[1,2] Timolol maleate (TM) in the form of eye drops is one of the most commonly used drugs in the treatment of open-angle glaucoma. An increase in the ocular bioavailability of TM eye drops is important for the treatment of glaucoma. This can be achieved by ensuring that eye drops do not drain and enhancing the residence time of eye drops.[3,4]

Many ophthalmic drugs are used at high doses or for longer periods to increase ocular bioavailability, but this increases the likelihood of causing ocular and systemic side effects.[5] Popular conventional ocular dosage forms, such as solution or suspension, have several limitations, notably a large drainage factor, short residence time, and poor bioavailability due to high tear fluid turnover.[1] Such causes usually result in an ocular bioavailability of less than 10%.[6] We are able to list the desired properties in an ophthalmic formulation as follows: be in the form of drops, not cause blurred vision or irritation, be able to withstand dilution of lacrimal fluid without rapid precorneal

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elimination after administration, and have a mucoadhesive property suitable for improving drug retention in the precorneal space.[6]

Different approaches, such as hydrogels, *in situ* gelling systems, microparticles, and colloidal carriers, are used to improve the therapeutic efficacy of ophthalmic pharmaceutical formulations and improve the bioavailability of administered drugs by increasing pre-corneal residence time and corneal penetration.[7] Gupta et al.[8] developed a temperature- and pH-triggered gel system using chitosan (CHT) and poloxamer 407. The formulation developed in this study showed significantly higher drug transport across the corneal membrane and increased ocular retention time. In the present study, different types (P338, P188) and ratios of poloxamers were tested, and characterization studies were conducted. Furthermore, the effects of mucoadhesive agents such as CHT and carboxymethylcellulose (CMC) were evaluated in terms of gelation time, viscosity, and mechanical properties. The effect of the gel formulations was demonstrated by *in vitro* release and *ex vivo* permeation compared with the commercial product in our study.[8]

Numerous studies have been conducted on systems based on solution gelling *in situ* using various polymers that undergo sol-gel phase transitions as a result of physical/chemical changes depending on pH, temperature, or a particular ion.[9-13] Poloxamers are thermosensitive, non-ionic polyoxyethylene-polyoxypropylene-polyoxyethylene (PEO n-PPO n-PEO n) tri-block copolymers.[14,15] It transforms from a low-viscosity solution to a gel at room temperature when its aqueous solution is 18% (*w/w*) or higher. Because the solution has a low poloxamer concentration, it loses its gelling ability after dilution with a lacrimal fluid, and this requires a higher poloxamer concentration [25% (*w/w*)]. In this case, the gelling temperature (GT) is lower than room temperature, and the solution must be stored in a refrigerator, making it difficult to prepare and use. Therefore, adding an analog of the poloxamer, for example, poloxamer 188 (P188), is a good alternative to increase the GT.[6]

The present study aim to prepare TM gel formulations containing poloxamer 338 (P338), poloxamer 188 (P188), and a mucoadhesive agent (CHT, CMC) to provide a sustained effect and a mucoadhesive thermosensitive formulation that gels in the eye when liquid at room temperature develops. It is aimed that the developed formulation is in the form of drops and can be applied easily, gels can be applied at eye temperature, and contact with the eye for a longer time. With its mucoadhesive feature, it is aimed to improve drug retention in the pre-corneal space. Thus, the drug stays in the eye for a longer time and increase its bioavailability.

MATERIALS AND METHODS

Materials

All chemicals and reagents used were of pharmaceutical and analytical grade. P338 and P188 were provided by BASF (Germany). CHT (low molecular weight) was purchased from Sigma-Aldrich (Steinheim, Germany), and CMC was provided by Aklar Chemistry (Ankara, Türkiye). The commercial product containing an equal amount of TM is a subsidiary of Bilim Pharmaceuticals (Türkiye). Active ingredient TM was obtained from Merck, USA.

Method

Preparation of gel formulations

Different concentrations of P338 and P188 were dissolved in cold isotonic water by mixing with a magnetic stirrer at 500 rpm in an ice bath for 20 minutes. The mucoadhesive agents (CHT, CMC) were added and dissolved. The formulations were refrigerated for at least 24 hours to ensure complete dissolution. Then, TM was added.

Drug content

Here, 0,5 g of the gel formulation was weighed and stirred in 100 mL of tear fluid for 24 hours at room temperature and 100 rpm on a magnetic stirrer. The TM concentration was measured at 295 nm using an ultraviolet (UV) spectrophotometer.[16]

pH determination

The pH values of the gel formulations were measured using a pH meter (Ohaus Corporatıion, USA) after being allowed to stand at room temperature for 1 hour. The pH of the developed gel formulations was adjusted to 7.4.

Viscosity measurement

Viscosity measurements of the gels were performed using a stress-controlled cone and plate rheometer (Brookfield, DV-III Rheometer). 0.5 mL sample was used and measurement was performed with spindle type CPE-52. The study was conducted in two different situations with and without the addition of an active ingredient, and three replicates were performed.

Determination sol-gel time

The test-tube inverting technique was used to determine the samples' sol-gel gelation times.[17] Briefly, a 10 mL test tube with a diameter of 1.0 cm was filled with 2 mL of the solution, and time measurements were started when the test tube was placed in a digital water bath at 34 °C. The flowability of the sample was monitored every 5 s by tilting the tubes. The gelation time was determined as the moment the samples' flow ceased, and the values were noted (Figure 1).

Texture profile analysis (TPA)

A TA-XT Plus Texture Analyzer (Stable Micro Systems, London, UK) was used to analyze the mechanical properties of the gels. The study was performed by attaching a penetrometer (moving probe) probe to the device. Approximately 50 mL of the gel formulation was placed in a beaker (100 mL). A pressure of 2 mm/s and a depth of 15 mm were applied twice on the gels with a 10 mm-diameter probe. The interval between the two compressions was adjusted to 15 s in each period.[18] Data obtained from Texture Exponent 2.0.6.0. calculated with a software. The mechanical properties of the gel formulations, including hardness, tackiness, cohesiveness, and elasticity, were determined.

In vitro release study

Franz diffusion cells were used in the *in vitro* release study and 0.5 mL of the formulation was transferred to the donor chamber. Artificial tear fluid was filled into the receptor chamber, which has a 2.5 mL volume, and stirred continuously using a small magnetic bar. The molecular weight of the dialysis membrane used to separate the donor and acceptor chambers was 12,000-14,000 Da. The experiment was conducted at 34 °C ± 2 °C. Samples were collected at specified time points (0.25 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, 10 hours, and 24 hours) and replaced with an equal volume of artificial tear fluid. Samples were analyzed by UV spectrophotometer at 295 nm (n= 3). The release profile was created by plotting the cumulative amount of TM released from the formulations over time. The artificial tear fluid's ingredients were filtered water (*q.s.* 100 g), sodium bicarbonate (0.220 g), sodium chloride (0.670 g), and calcium chloride dehydrate (0.008 g). This fluid was used to simulate tear fluid.[8]

Ex vivo permeability study

Ex vivo experiments were performed using corneas from cow eyeballs collected from slaughterhouses immediately after the animal was sacrificed.[19] The cornea was carefully removed with 4-5 mm of surrounding scleral tissue. The removed corneas were cleaned with cold saline and stored in fresh simulated tear fluid before use (Figure 2).[20] The corneas were placed between the donor and receptor compartments of the Franz diffusion cell, with the endothelium facing the receptor compartment. The temperature was maintained constant at 34 ± 2 °C throughout the experiment. A volume of 0.5 mL of each formulation was administered, and the experiment was performed in triplicate (n= 3). Subsequently, the same procedures used in the *in vitro* release study were applied to the *ex vivo* permeation study. Permeated amount of TM (%) from the cow cornea versus time was plotted.

Statistical analysis

GraphPad Prism 5.0 Software was used in statistical analyses. One-Way ANOVA test was used for group comparisons. In the analyses, a significant difference was determined as $P < 0.05$.

RESULTS

Gelation time study

Different combinations of P338, P188, CMC, and CHT were studied to prepare the mucoadhesive thermosensitive gel formulations. The gelling times were found to be 26.67 seconds for the CHT gel and 35 seconds for the CMC gel to be the most suitable (Table 1).

Viscosity measurement

Viscosity measurements of the ophthalmic gel formulations were performed with and without TM. While the viscosity of the CHT gel formulation was 1572.66 mPa.s, that of the CMC gel

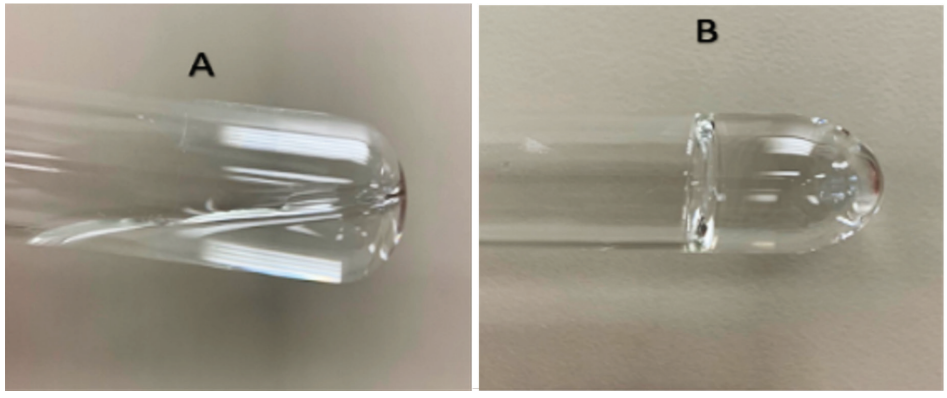

Figure 1. The figure shows the gels in solution form at 25 °C (A) and transformed into gels at 34 °C (B)

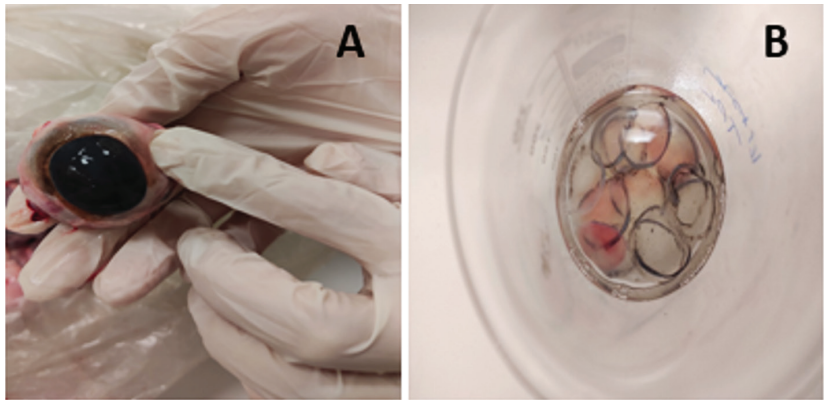

Figure 2. Freshly harvested cow eyes (A) and removed corneas (B)

formulation was 915 mPa.s, and the viscosity values increased with the addition of the drug. The gel formulation rheograms showed Newtonian fluid behavior at 25 °C when shear stress was plotted against shear rate (Figure 3).

Texture profile analysis

The mechanical properties of CHT and CMC gels were examined by texture profile analysis. The hardness, adhesiveness, cohesiveness and elasticity values obtained at the end of the study are given in Table 2. According to the texture profile analysis results, the mechanical properties of both systems were found to be similar.

In vitro release and ex vivo permeation studies

The *in vitro* drug release profiles of the CMC gel, CHT gel, commercial product, and TM solution are shown in Figure 4A. At the end of 24 hours, drug release was obtained at approximately the same percentage for the gels. At the end of 24 hours, TM released from the commercial products comprised 82% of the dose.

Figure 4B displays the results of *ex vivo* permeation tests. The amount of TM penetrating the cow cornea after 24 hours was 73.38%, 71.80%, 67.25%, and 60.55% from the CHT gel, CMC gel, TM solution, and commercial preparation, respectively.

Table 1. Gelation times of different *in situ* ophthalmic gel formulations at 34 °C

System	P338:188 (*w/v* %)	CMC (*w/v* %)	CHT (*w/v* %)	Gelation time (second)
CMC gel	18:2.5	0.1	-	98.33
	20:2.5	0.1	-	35
	22:2.5	0.1	-	gel
CHT gel	18:2.5	-	0.1	68.33
	20:2.5	-	0.1	26.67
	22:2.5	-	0.1	gel

CMC: Chitosan, CHT: Carboxymethylcellulose

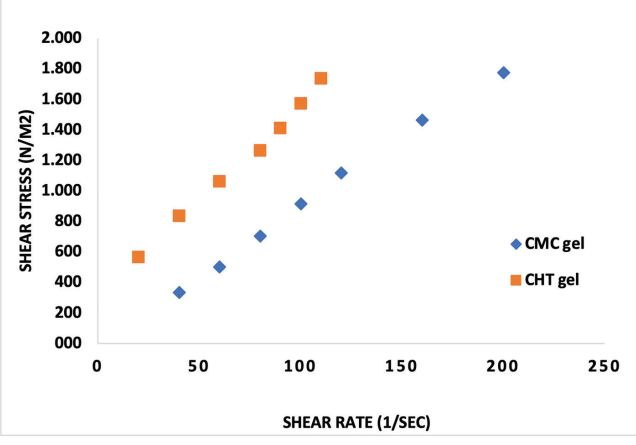

Figure 3. Viscosity measurements of the formulations at 25 °C
CMC: Carboxymethylcellulose, CHT: Chitosan

DISCUSSION

Poloxamers with thermogenic properties are non-ionic triblock copolymers consisting of one unit of polypropylene and two units of polyoxyethylene. The gelation process depends on the critical micelle concentration and temperature. At physiological temperatures and concentrations, the aqueous solution returns to a gel state *in situ*.

Different poloxamers can be used together to adjust the GT. Mucoadhesive polymer-and poloxamer-containing gels are systems that are being studied extensively as drug delivery systems. Poloxamer-based mucoadhesive gels have two main advantages: they have both gelation and mucoadhesion properties at physiological temperatures. The presence of a mucoadhesive polymer in an aqueous poloxamer solution may change the sol-gel transition temperature, gelation time, rheological properties, and release properties of the active substance. Similarly, the addition of poloxamer may affect the mucoadhesive properties of the mucoadhesive gel.[21]

In the present study, a poloxamer-based (two different types combined) mucoadhesive thermosensitive system was investigated. The aim is to prepare thermogel systems whose GTs are close to the corneal temperature. A combination of different poloxamers at different concentrations was used for suitable temperatures. According to the literature, the preferred mucoadhesive polymers, CHT and CMC, act as penetration enhancers to promote the drug's transcorneal permeability.[8]

Additionally, poloxamer, which are marketed as pluronic, have all the characteristics-including good thermal gelling, non-irritating eyes, and tolerance-that make them acceptable for ocular administration.[22]

The retention time of poloxamer-based gels is directly influenced by their mechanical and rheological characteristics. In the case of weak mechanical strength and low viscosity, rapid elimination occurs, whereas in the case of high viscosity, gel flow becomes problematic.[23] To evaluate these properties, we performed gelling time, viscosity and TPA analyses.

It was found that the polymer composition affected the physicochemical properties of *in situ* gel formulations, including TM. The formulation's Tsol-gel reduced when the P338 content was increased. The difference in the mucoadhesive agent also affected the gelation time. Soriano-Ruiz et al.[24] reported the preparation gels using different ratios of poloxamer to CHT and measured the gelation times. The gelling time was 1.16 min when 20% poloxamer/0.5 CHT was used and 0.86 minute when the poloxamer ratio was 22%. Gratieri et al.[25] assessed the gelation temperatures of gels using 16% poloxamer and 0.5%, 1%, and 1.5% CHT as 33 ± 0.8 °C, 32 ± 1.7 °C, 31 ± 1.3 °C, respectively. Morsi et al.[26] developed a ketorolac tromethamine-loaded thermosensitive *in situ* gel system for the treatment of postoperative ocular inflammation. Different concentrations of poloxamer and hydroxypropyl methylcellulose were used in the gel systems. Similar to our study, it was determined that as the poloxamer concentration increased, the gelation time and gelation temperature decreases.[26]

Table 2. Mechanical properties of the gel formulations

	Hardness (g) ± SD	Adhesiveness (g.second) ± SD	Cohesiveness ± SD	Elasticity ± SD
CHT gel	19.349 ± 0.452	-16.097 ± 0.179	0.882 ± 0.016	0.559 ± 0.014
CMC gel	20.857 ± 1.482	-14.999 ± 2.982	0.916 ± 0.047	0.544 ± 0.025

CMC: Chitosan, CHT: Carboxymethylcellulose, SD: Standard deviation

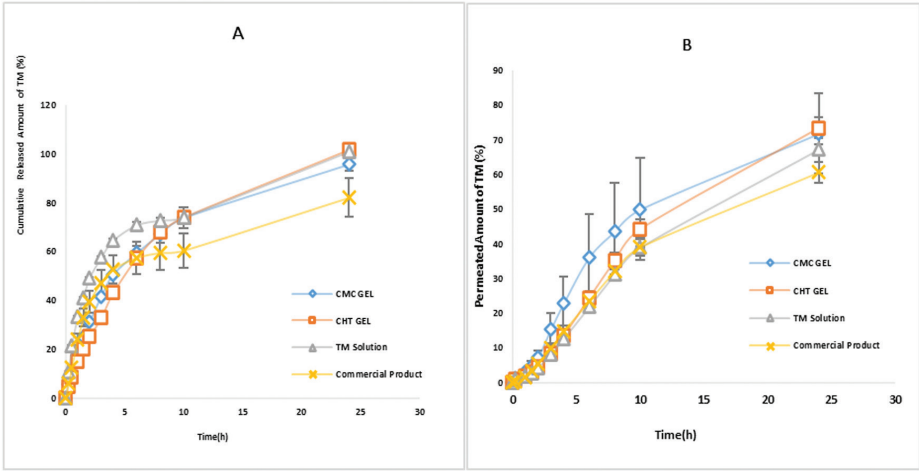

Figure 4. TM release and permeation profiles from TM-containing in situ ophthalmic gels through dialysis membranes (A) and cow corneas (B) ($p > 0.05$)
TM: Timolol maleate

As a result of viscosity studies performed at 25 °C, CHT and CMC gels show Newtonian fluid. Similar to our study, Tirnaksiz et al.[21] in their study, they concluded that the poloxamer solutions they prepared at different concentrations (10%, 12.5% and 15%) showed Newtonian fluid at three temperatures (25 °C, 30 °C and 35 °C).

In order to examine the mechanical properties of the gels, hardness, adhesiveness, cohesiveness and elasticity values were obtained. The amount of work needed to separate the probe from the formulation was defined by the adhesiveness value, which is related to the adhesive characteristics. The aim is to increase the residence time of the drug in the eye; therefore, a high adhesive value indicates stronger adhesion on the surface. According to the experimental results, the CHT gel formulation, which also exhibits higher gel strength properties, achieved the maximum adhesiveness. Cohesiveness indicates the difficulty in breaking down the internal structure of the gel and the effect of repeated stresses. The elasticity feature is that the gel is structurally restored after compression and deformation. A low numerical value indicates high product flexibility.[27] Hardness is defined as the force required to achieve a certain deformation, and it refers to the applicability of the gel to the desired area.[28] It is desirable to have low hardness values for the formulations so that they can be easily taken from the container and applied to the mucosal area.[29] In our study, the hardness of the CHT gel was lower than that of the CMC gel. It has been stated in the literature that there is a correlation between viscosity and hardness.[30] We found that the hardness value of the CHT gel, which has a lower viscosity, was also lower than that of the CMC gel, in line with the literature. Higher adhesive properties in CHT gels are important parameters in mucoadhesive gel design because better gel contact and retention will improve clinical efficacy.

The *in vitro* release properties of CMC gel, CHT gel, commercial product and TM solution were investigated using dialysis membrane. As expected, there was a faster release in the TM solution in the first hours compared with the other formulations, and the release rate was lower for the gel systems. The release of the drug from the gels and TM solution was characterized by an initial phase of high release (burst effect).[31]

The cumulative percentage of drug release through the corneal membrane was slightly lower than that through the dialysis membrane. This might be because the dialysis membrane acts as a basic mechanical barrier, whereas the cornea's epithelium, stroma, and endothelium act as a lipophilic hydrophilic barrier for corneal penetration.[31]

Although the difference between the groups was found to be statistically insignificant as a result of the *ex vivo* permeation study ($p > 0.05$), the permeability of the gel formulation was higher than the solution and commercial product. Owing to its thermosensitive, mucoadhesive gel formulations, TM penetrated the cornea more effectively for a longer period. Thermosensitive *in situ* hydrogels may increase ocular bioavailability by prolonging drug release.[32]

In our study, *in vitro* release and *ex vivo* permeability studies of the developed gels were performed and compared with gel formulations, solutions and commercial products. Thus, it was observed that TM gel systems prolonged the release and increased the retention time in the eye. In particular, different types of poloxamer (P338 and P188) have been used

in combination instead of the poloxamer 407 used in many studies. At the same time, different gel formulations have been developed with the addition of mucoadhesive agents.[25,33-35]

In another study, an ocular gel system sensitive to temperature and pH was developed using poloxamer and CHT polymers. In the *in vitro* transcorneal permeability study, the gel system developed with the drug solution was compared. After 4 hours, the permeability of the drug through the goat cornea was 42.11% ± 2.1% for the solution and 63.41% ± 2.6% for the gel system. This situation was interpreted as being explained by the good transmucosal enhancer properties of CHT.[8]

Similar to our study, one study concluded that an ophthalmic gel developed *in situ* gel showed significantly improved bioavailability compared with a commercial aqueous solution. The developed *in situ* gel formulation was noted to show potential for use as a delivery system for carteolol hydrochloride with superior ocular bioavailability.[36] Gratieri et al.[25] in their study developed a thermosensitive gel formulation by experimenting with different ratios of poloxamer to CHT. The results showed that CHT improved the mechanical strength and tissue properties of the poloxamer formulations. It has been reported that the poloxamer/CHT gel makes contact with the corneal surface four times more than a conventional solution. It was concluded that the developed *in situ* shaping gel is a promising tool for the topical treatment of ocular diseases.

The ocular bioavailability and retention time of TM can be increased with *in situ* gel formulations prepared using polymers other than those used in this study, such as carbopol, polycarbophil, cellulose acetophthalate latex, gellan gum, alginate, ethyl (hydroxyethyl) cellulose, methylcellulose, and Smart Hydrogel™. It can be suggested that the effectiveness of the developed gel formulations can be supported by *in vivo* studies.

CONCLUSION

In this study, thermosensitive-mucoadhesive ophthalmic gel formulations were developed using different types and concentrations of poloxamers and mucoadhesive polymers. The developed gel formulations are liquid at room temperature, and when applied to the cornea, they can form in a short time, such as 26.67 (CHT gel) or 35 seconds (CMC gel). Mucoadhesive and thermosensitive polymer types and concentrations affect gelation time. In the TPA analysis, the hardness value of the CHT gel was found to be lower than that of the CMC gel in relation to viscosity. As a result of *in vitro* release, faster drug release was observed in the TM solution compared with the other formulations in the first hours, but at the end of 24 hours, approximately the same percentage of drug release was obtained with the gels. According to an *ex vivo* permeation study, TM penetrated the cornea longer and more effectively because of the thermosensitive, mucoadhesive gel formulations. The developed gel systems effectively controlled the release of relatively hydrophilic drugs, such as timolol maleate, compared to eye drops and increased drug permeability when using mucoadhesive polymers. Thermosensitive-mucoadhesive hydrogels are effective systems for increasing ocular bioavailability and reducing the frequency of drug application by prolonging drug release. It was concluded that the developed gel formulations are better in contact with the corneal surface than a commercial solution, making them a promising tool for the topical treatment of ocular diseases and improving patient compliance.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: There is not any animal or human experiment in our study.

Authorship Contributions

Surgical and Medical Practices: Ö.K., E.T.A., S.I-T. Concept: Ö.K., E.T.A., S.I-T. F.F.T., Design: Ö.K., E.T.A., S.I-T. F.F.T., Data Collection or Processing: Ö.K., E.T.A., S.I-T. F.F.T., Analysis or Interpretation: Ö.K., E.T.A., S.I-T. Literature Search: Ö.K., E.T.A., S.I-T. F.F.T., Writing: Ö.K., E.T.A., S.I-T. F.F.T.

Conflict of Interest: The authors declare that they have no conflict of interest.

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