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

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Abstract

Objectives: The purpose 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 mucoadhesive agents in cold isotonic water with a magnetic stirrer. The sol-gel gelation time of the gels was determined using the test tube inversion method. Viscosity measurements and analysis of mechanical properties of gel formulations were carried out. In vitro release using dialysis membrane and ex vivo permeation studies using fresh cow eyes were performed.

Results: The gelation times of the formulations containing 20:2.5 (P338:P188) and 0.1% CMC and formulations containing 20:2.5 (P338:P188) and 0.1% CHT were found to be 35 seconds and 26.67 seconds, respectively. 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 flow, and the viscosity of the CHT gel was found to be higher. CHT gel showed better mechanical properties than 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 can successfully control the release of TM. The significantly lower drainage of TM into the circulation compared to eye drops provides an advantage in the treatment of 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, which can cause irreversible blindness if left undiagnosed and untreated.[1,2] TM in the form of eye drops is one of the most used drugs in the treatment of open-angle glaucoma. Increase of the ocular bioavailability of TM eye drops is very important for the treatment of glaucoma. This can be achieved by ensuring not draining 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 large drainage factor, short residence time, and poor bioavailability due to the 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 elimination after administration, 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, improving the bioavailability of administered drugs by increasing pre-corneal residence time and corneal penetration.[7] Gupta et al. developed a temperature and pH-triggered gel system using chitosan and poloxamer 407. The formulation developed similar to our study showed significantly higher drug transport across the corneal membrane and increased ocular retention time. In our present study, different types (P338, P188) and ratios of poloxamer were tested and characterization studies were carried out. Furthermore, the

effect of remarkable mucoadhesive agents such as chitosan and CMC were evaluated in terms of gelation time, viscosity and mechanical properties of the gel. The effect of gel formulations was demonstrated by in vitro release and ex vivo permeation results compared with the commercial product in our study.[8]

Numerous studies have been conducted on systems based on a solution gelling in situ using various polymers that undergo sol-gel phase transitions as a result of physical/chemical change 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 at a concentration of 18% (w/w) or higher. Since the solution has a low poloxamer concentration, it loses its gelling ability after dilution with lacrimal fluid, and this requires a higher concentration (25% (w/w)). In this case, the gelling temperature (GT) will be lower than room temperature and the solution must be stored in the refrigerator, making it difficult to prepare and use. Therefore, adding an analog of poloxamer, for example poloxamer 188 (P188), is a good alternative to increase the GT.[6]

The aim of the present study was to prepare TM gel formulations containing poloxamer 338 (P338), poloxamer 188 (P188), and mucoadhesive agents (chitosan (CHT), carboxymethylcellulose (CMC)) to provide a sustained effect and a mucoadhesive-thermosensitive formulation that gels in the eye when liquid at room temperature is to develop. It is aimed that the developed formulation is in the form of drops and can be applied easily, gels at eye temperature, and contacts the eye for a longer time, and with its mucoadhesive feature, it is aimed to improve drug retention in the pre-corneal space. Thus, the drug will stay 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) purchased from Sigma Aldrich (Steinheim, Germany) and CMC provided by Aklar Chemistry (Ankara,Turkey). The commercial product containing an equal amount of the TM belongs to Bilim Pharmaceuticals (Turkey). Active ingredient TM was from Merck, USA.

Method

Preparation of Gel Formulations

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

Drug content

Here, 0,5 g of 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. TM concentration was measured at 295 nm using UV spectrophotometer.[16]

pH determination

The pH values of the gel formulations were measured with a pH meter (Ohaus Corporatıon,USA) after standing at room temperature for 1 hour. The pH of the developed gel formulations was adjusted to 7.4.

Measurement of Viscosity

Viscosity measurements of the gels were measured using a stress-controlled cone and a plate rheometer (Brookfield, DV-III Rheometer). 0.5mL sample was used and measurement was performed with spindle type CPE-52. The study was performed in two different situations with and without the added 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 observed every 5 sec by tilting the tubes. The gelation time was determined as the moment the samples' flow ceased, and the values were noted.

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 carried out 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/sec and a depth of 15 mm was applied twice on the gels with a 10 mm diameter probe. It was adjusted to be 15 seconds between two compressions in each period.[18] Data obtained Texture Exponent 2.0.6.0. calculated with a software program. The mechanical properties of the gel formulations, including hardness, tackiness, cohesiveness, and elasticity, were determined.

In Vitro Release Study

Franz diffusion cells were used for 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 the tiny magnetic bar. The molecular weight of the dialysis membrane used to separate the donor and acceptor chambers is 12,000–14,000 Da. The experiment was carried out at 34°C ± 2°C. Samples were taken at the 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 Permeation Study

Ex vivo experiments were performed using cow cornea from the cow eyeball collected from the slaughter house 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 simulate tear fluid before use.[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 kept constant at 34 ± 2 °C throughout the experiment. 0.5 mL of each formulation was administered and the experiment was performed in triplicate (n=3). Subsequently, same procedures used in the in vitro release study were also applied to the ex vivo permeation study. Permeated amount of TM (%) from cow cornea versus time was plotted.

RESULTS AND DISCUSSION

Poloxamers having thermo-gelling properties are non-ionic triblock copolymers consisting of one unit of polyoxypropylene and two units of polyoxyethylene units. The gelation process depends on critical micelle concentration and critical micelle temperature. At the physiological temperature and certain concentrations, an aqueous solution of returns to a gel state in situ.

Different poloxamers can be used together to adjust the gelling temperature. 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: having both gelation and mucoadhesion properties at the physiological temperature. The presence of the 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 property of the mucoadhesive gel.[21]

In the presented study, poloxamer based (two different types combined) mucoadhesive thermosensitive system was investigated. The aim is to prepare thermogel systems whose gelling temperature is close to the corneal temperature. Combination of different poloxamers in different concentrations was used for suitable temperatures. According to literature, the study's preferred mucoadhesive polymers, CHT and CMC, act as penetration enhancers to promote the drug's transcorneal permeability.

Additionally, poloxamers, which are marketed as pluronic, have all the characteristic - 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 case of weak mechanical strength and low viscosity, rapid elimination occurs, while in case of high these values, gel flow becomes problematic.[23] To evaluate these properties, we performed viscosity and TPA analyses in this study.

Gelation Time Study

Different combinations of P338, P188, CMC and CHT were studied to prepare the mucoadhesive-thermosensitive gel formulations. It was found that the polymer compositions had an impact on the physicochemical properties of in situ gel formulations including TM. For instance, the formulation's Tsol-gel reduced when the P338 amount was increased. Also, the difference in the mucoadhesive agent also changed the gelation time. The gelling times were found to be 26.67 sec for the CHT gel and 35 sec for the CMC gel as the most suitable (Table 1). Soriano-Ruiz et al.[24] reported the preparation gels using different ratios of poloxamer and chitosan and measured their gelation times. The gelling time was 1.16 minutes when 20% poloxamer 0.5% chitosan was used, and 0.86 minutes when the poloxamer ratio was 22%. As the poloxamer concentration increased at certain concentrations, the gelation time was shortened. Gratieri et al.[25] found the gelation temperatures of gels prepared using 16% poloxamer and 0.5%, 1%, 1.5% chitosan as 33 ± 0.8°C, 32 ± 1.7°C, 31 ± 1.3°C, respectively. Morsi et al. developed a ketorolac tromethamine loaded thermosensitive in situ gel system for the treatment of postoperative ocular inflammation. Different concentrations of poloxamer and HPMC were used in gel systems. Similar to our study, it was determined that as the poloxamer concentration increases, the gelation time and gelation temperature decreases.[26]

Measurement of Viscosity

Viscosity measurements of the ophthalmic gel formulations were obtained with and without TM. While the viscosity of the CHT gel formulation was 1572.66 mPa.s, the CMC gel formulation was found to be 915 mPa.s and the viscosity values increased with the addition of drug. The rheograms of gel formulations showed Newtonian flow behavior at 25°C when shear stress was plotted against shear rate (Figure 3). Similar to our study, Tırnaksiz et al.[21] in their study, they concluded that the poloxamer solutions they prepared at different concentrations (10%, 12.5% and 15%) showed Newtonian flow at three temperatures (25°C, 30°C and 35 °C).

Texture Profile Analysis

According to the Texture Profile analysis results, the mechanical properties of both systems were found to be similar (Table 2). The amount of work needed to separate the probe from the formulation is defined by the adhesiveness value, which is related to adhesive characteristics. The aim is to enhance the drugs retention time, hence a higher adhesiveness quantity suggests stronger adhesion at the surface. According to the experiment's results, the CHT gel formulation, which also exhibits higher gel strength properties, achieved the maximum

adhesiveness value. Cohesiveness indicates the difficulty in breaking down the internal structure of the gel and the effect of repeated stresses. 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 refers to the applicability of the gel to the desired area.[28] It is desirable that the hardness values of the formulations be low so that the formulation can be easily taken from the container and applied to the mucosal area.[29] In our study, the hardness value of the CHT gel is lower than 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 found to be lower than the CMC gel, in line with the literature.

Higher adhesive property in CHT gel is an important parameter in mucoadhesive gel design, as better gel contact and retention will provide better clinical efficacy.

In Vitro Release and Ex Vivo Permeation Study

The in vitro drug release profile of CMC gel, CHT gel, commercial product and TM solution is shown in Figure 4A. As expected, there was a faster release in the TM solution in the first hours compared to other formulations, and the release rate was less for gel systems. The release of drug from the gels and TM solution was characterized by an initial phase of high release (burst effect).31 The end of 24 hours, drug release was obtained at approximately at the same percentage for the gels. Whereas at the end of 24 hours, TM released from the commercial products was 82% of the dose.

Figure 4B displays the results of the ex vivo permeation tests. The cumulative percentage of drug release through the cow corneal membrane was slightly less compared to the dialysis membrane. This might be because the dialysis membrane just acts as a basic mechanical barrier, whereas the cornea's epithelium, stroma, and endothelium act as a lipophilic hydrophilic barrier for corneal penetration.[31] 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. Although the difference was found to be statistically insignificant ($p>0.05$, one way ANOVA), the permeability of gel formulations is higher than that of the solution and commercial product. Thanks to its thermosensitive mucoadhesive gel formulations, TM penetrated the cornea more effective for a longer period. Thermosensitive in situ hydrogels may increase ocular bioavailability by prolonging drug release.[32]

In vitro release of the gels developed in our study, ex vivo permeation study was performed and compared with gel formulations, solution and commercial product. Thus, it has been observed that the TM gel systems delay the release and prolongs the retention time in the eye. In particular, different types of poloxamers (P338 and P188) have been used in combination instead of poloxamer 407 used in many literatures. 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 that sensitive to temperature and pH was developed using poloxamer and chitosan 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 is 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 chitosan.[8]

Similar to our study, one study concluded that the ophthalmically developed in situ gel showed significantly improved bioavailability compared to a commercial aqueous solution. The developed in situ gel formulation was noted to show potential for use as delivery systems of carteolol HCl with superior ocular bioavailability.[36] Gratieri et al.[25] in their study, they developed a thermosensitive gel formulation by experimenting with different ratios of

poloxamer and chitosan. The results showed that chitosan improved the mechanical strength and tissue properties of poloxamer formulations. It has been reported that the developed poloxamer/chitosan gel is in contact with the corneal surface four times more than a conventional solution. It is concluded that the developed in-situ shaping gel is a promising tool for the topical treatment of ocular diseases.

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

CONCLUSION

In this study, thermosensitive-mucoadhesive ophthalmic gel formulations were developed by using different types and concentrations of poloxamer and mucoadhesive polymers. The developed gel formulations are liquid at room temperature, and when applied to the cornea, they can gel form in a short time such as 26.67 (CHT gel) or 35 seconds (CMC gel). Mucoadhesive and thermosensitive polymer type and concentration affect the gelation time. In the TPA analysis, the hardness value of the CHT gel was found to be lower than the CMC gel in relation to the viscosity. As a result of in vitro release, faster drug release was observed in the TM solution compared to 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 the ex vivo permeation study, TM penetrated the cornea longer and more effectively thanks to 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 with mucoadhesive polymers. Thermosensitive-mucoadhesive hydrogels are effective systems for increasing ocular bioavailability and reducing the frequency of 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 it a promising tool for the topical treatment of ocular diseases and better patient compliance.

REFERENCES

1. Khattab A, Marzok S, Ibrahim M. Development of optimized mucoadhesive thermosensitive pluronic based in situ gel for controlled delivery of Latanoprost: Antiglaucoma efficacy and stability approaches. J Drug Deliv Sci Technol [Internet]. 2019;53(June):101134. Available from: https://doi.org/10.1016/j.jddst.2019.101134
2. Lee DA, Higginbotham EJ. Glaucoma and its treatment: A review. Am J Heal Pharm. 2005;62(7):691–9.
3. Kamel AH El. In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate. Int J Pharm. 2002;241:47–55.
4. Aggarwal D, Kaur IP. Improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system. Int J Pharm. 2005;290(1–2):155–9.
5. Urtti A, Rouhiainen H, Kaila T, Saano V. Controlled Ocular Timolol Delivery: Systemic Absorption and Intraocular Pressure Effects in Humans. Vol. 11, Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists. 1994. p. 1278–82.

6. Qi H, Chen W, Huang C, Li L, Chen C, Li W. Development of a poloxamer analogs/carbopol-based in situ gelling and mucoadhesive ophthalmic delivery system for puerarin. Int J Pharm. 2007;337(1–2):178–87.
7. Almeida H, Amaral MH, Lobão P, Silva AC, Lobo JMS. Applications of polymeric and lipid nanoparticles in ophthalmic pharmaceutical formulations: Present and future considerations. J Pharm Pharm Sci. 2014;17(3):278–93.
8. Gupta H, Jain S, Mathur R, Mishra P, Mishra AK, Velpandian T. Sustained ocular drug delivery from a temperature and pH triggered novel in situ gel system. Drug Deliv. 2007;14(8):507–15.
9. Zhu M, Wang J, Li N. A novel thermo-sensitive hydrogel-based on poly(N-isopropylacrylamide)/hyaluronic acid of ketoconazole for ophthalmic delivery. Artif Cells, Nanomedicine Biotechnol. 2018;46(6):1282–7.
10. Allam A, Elsabahy M, El Badry M, Eleraky NE. Betaxolol-loaded niosomes integrated within pH-sensitive in situ forming gel for management of glaucoma. Int J Pharm [Internet].2021;598(February):120380.Availablefrom:https://doi.org/10.1016/j.ijpharm.2021.120380
11. Hsiue GH, Hsu SH, Yang CC, Lee SH, Yang IK. Preparation of controlled release ophthalmic drops, for glaucoma therapy using thermosensitive poly-N-isopropylacrylamide. Biomaterials. 2002;23(2):457–62.
12. Zhu Q, Cheng H, Huo Y, Mao S. Sustained ophthalmic delivery of highly soluble drug using pH-triggered inner layer-embedded contact lens. Int J Pharm [Internet]. 2018;544(1):100–11. Available from: https://doi.org/10.1016/j.ijpharm.2018.04.004
13. Ho AY, Olm-Shipman M, Zhang Z, Siu CT, Wilgucki M, Phung A. A Randomized Trial of Mometasone Furoate 0.1% to Reduce High-Grade Acute Radiation Dermatitis in Breast Cancer Patients Receiving Postmastectomy Radiation. Int J Radiat Oncol Biol Phys. 2018 Jun 1;101(2):325–33.
14. Çulcu Ö, Tunçel E, IIbasmis-Tamer S, Tirnaksiz F. Journal of Gazi University Health Sciences Institute. J Gazi Univ Heal Sci Inst. 2021;2(December):28–44.
15. Almeida H, Lobão P, Frigerio C, Fonseca J, Silva R, Quaresma P. Development of mucoadhesive and thermosensitive eyedrops to improve the ophthalmic bioavailability of ibuprofen. J Drug Deliv Sci Technol. 2016;35:69–80.
16. Gupta, S., & Vyas, S. P. Carbopol/chitosan based pH triggered in situ gelling system for ocular delivery of timolol maleate. Sci Pharm. 2010;78(4), 959-976.
17. Chen X, Zhi F, Jia X, Zhang X, Ambardekar R, Meng Z. Enhanced brain targeting of curcumin by intranasal administration of a thermosensitive poloxamer hydrogel. J Pharm Pharmacol. 2013;65(6):807–16.
18. Tuğcu-Demiröz, F., Acartürk, F., & Özkul, A. Preparation and characterization of bioadhesive controlled-release gels of cidofovir for vaginal delivery. J Biomater Sci. 2015;26(17), 1237–1255.
19. Barse R, Kokare C, Tagalpallewar A. Influence of hydroxypropylmethylcellulose and poloxamer composite on developed ophthalmic in situ gel: Ex vivo and in vivo characterization [Internet]. Vol. 33, Journal of Drug Delivery Science and Technology. Elsevier Ltd; 2016. 66–74 p. Available from: http://dx.doi.org/10.1016/j.jddst.2016.03.011
20. Kouchak M, Mahmoodzadeh M, Farrahi F. Designing of a pH-Triggered Carbopol®/HPMC In Situ Gel for Ocular Delivery of Dorzolamide HCl: In Vitro, In Vivo, and Ex Vivo Evaluation. AAPS Pharm.Sci. Tech. 2019;20(5):1–8.

21. Tirnaksiz F, Robinson JR. Rheological, mucoadhesive and release properties of Pluronic F-127 gel and Pluronic F-127/polycarbophil mixed gel systems. Pharmazie. 2005;60(7):518–23.
22. Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J. Research progress of in-situ gelling ophthalmic drug delivery system. Asian J Pharm Sci. 2019;14(1):1–15.
23. Soliman KA, Ullah K, Shah A, Jones DS, Singh TRR. Poloxamer-based in situ gelling thermoresponsive systems for ocular drug delivery applications. Drug Discov Today. 2019 Aug;24(8):1575–86.
24. Soriano-Ruiz JL, Calpena-Campmany AC, Silva-Abreu M, Halbout-Bellowa L, Bozal-de Febrer N, Rodríguez-Lagunas MJ. Design and evaluation of a multifunctional thermosensitive poloxamer-chitosan-hyaluronic acid gel for the treatment of skin burns. Int J Biol Macromol. 2020;142:412–22.
25. Gratieri T, Gelfuso GM, Rocha EM, Sarmento VH, de Freitas O, Lopez RFV. A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery. Eur J Pharm Biopharm [Internet]. 2010;75(2):186–93. Available from: http://dx.doi.org/10.1016/j.ejpb.2010.02.011
26. Morsi, N., Ghorab, D., Refai, H., & Teba, H. Ketoroloac tromethamine loaded nanodispersion incorporated into thermosensitive in situ gel for prolonged ocular delivery. Int J Pharm.2016;506(1-2), 57-67.
27. Ustundag Okur N, Yozgatli V, Senyigit Z. Formulation and detailed characterization of voriconazole loaded in situ gels for ocular application. Ankara Univ Eczac Fak Derg. 2020;44(1):33–49.
28. Jones DS, Woolfson AD, Brown AF. Textural analysis and flow rheometry of novel, bioadhesive antimicrobial oral gels. Vol. 14, Pharmaceutical Research. 1997. p. 450–7.
29. Rençber S, Karavana SY, Şenyiğit ZA, Eraç B, Limoncu MH, Baloğlu E. Mucoadhesive in situ gel formulation for vaginal delivery of clotrimazole: formulation, preparation, and in vitro/in vivo evaluation. Pharm Dev Technol. 2017;22(4):551–61.
30. Tuğcu-Demiröz F, Acartürk F, Erdoğan D. Development of long-acting bioadhesive vaginal gels of oxybutynin: Formulation, in vitro and in vivo evaluations. Int J Pharm. 2013;457(1):25–39.
31. Sawant D, Dandagi PM, Gadad AP. Formulation and evaluation of sparfloxacin emulsomes-loaded thermosensitive in situ gel for ophthalmic delivery. J Sol-Gel Sci Technol. 2016;77(3):654–65.
32. Huang W, Zhang N, Hua H, Liu T, Tang Y, Fu L. Preparation, pharmacokinetics and pharmacodynamics of ophthalmic thermosensitive in situ hydrogel of betaxolol hydrochloride. Biomed Pharmacother [Internet]. 2016;83:107–13. Available from: http://dx.doi.org/10.1016/j.biopha.2016.06.024
33. Gratieri, T., Gelfuso, G. M., de Freitas, O., Rocha, E. M., & Lopez, R. F. Enhancing and sustaining the topical ocular delivery of fluconazole using chitosan solution and poloxamer/chitosan in situ forming gel. Eur J Pharm Biopharm. 2011; 79(2), 320-327
34. Varshosaz, J., Tabbakhian, M., & Salmani, Z. Designing of a thermosensitive chitosan/poloxamer in situ gel for ocular delivery of ciprofloxacin. The Open Drug Delivery J. (2008);2(1).
35. Edsman, K., Carlfors, J., & Petersson, R. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur J Pharm Sci. (1998); 6(2), 105-112.
36. El-Kamel A, Al-Dosari H, Al-Jenoobi F. Environmentally responsive ophthalmic gel formulation of carteolol hydrochloride. Drug Deliv J Deliv Target Ther Agents. 2006;13(1):55–9.

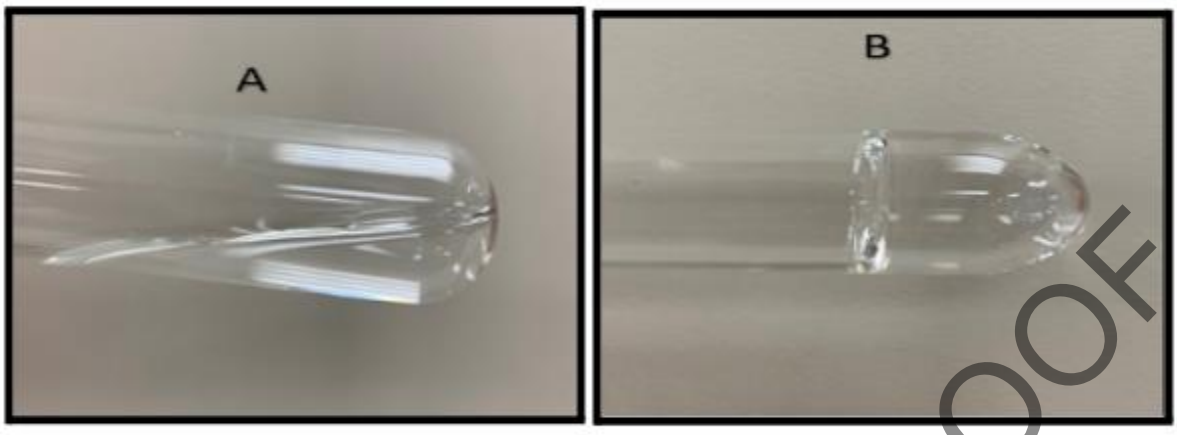

Figure 1. The figure shows the gels in solution form at 25 °C (A) and their transformed into gels at 34 °C (B)

Figure 2. Freshly harvested cow's eye (A) and removed corneas (B)

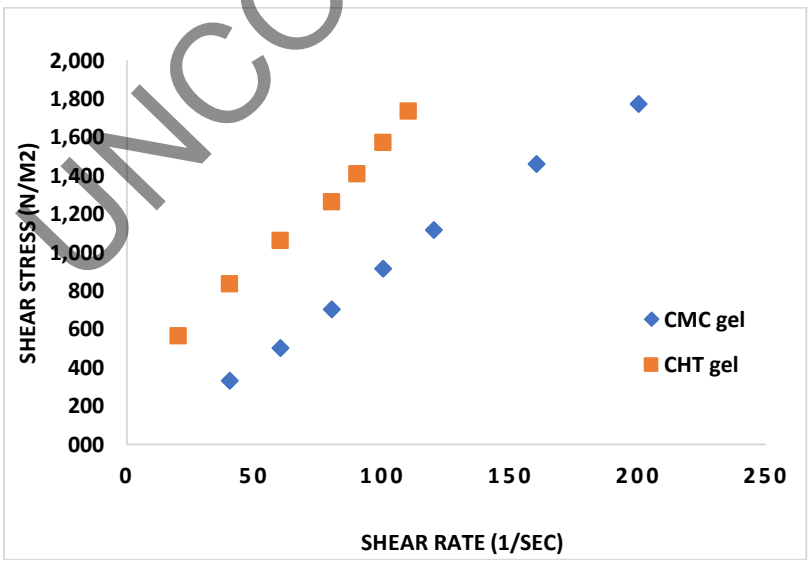

Figure 3. Viscosity measurements of formulations at 25 °C

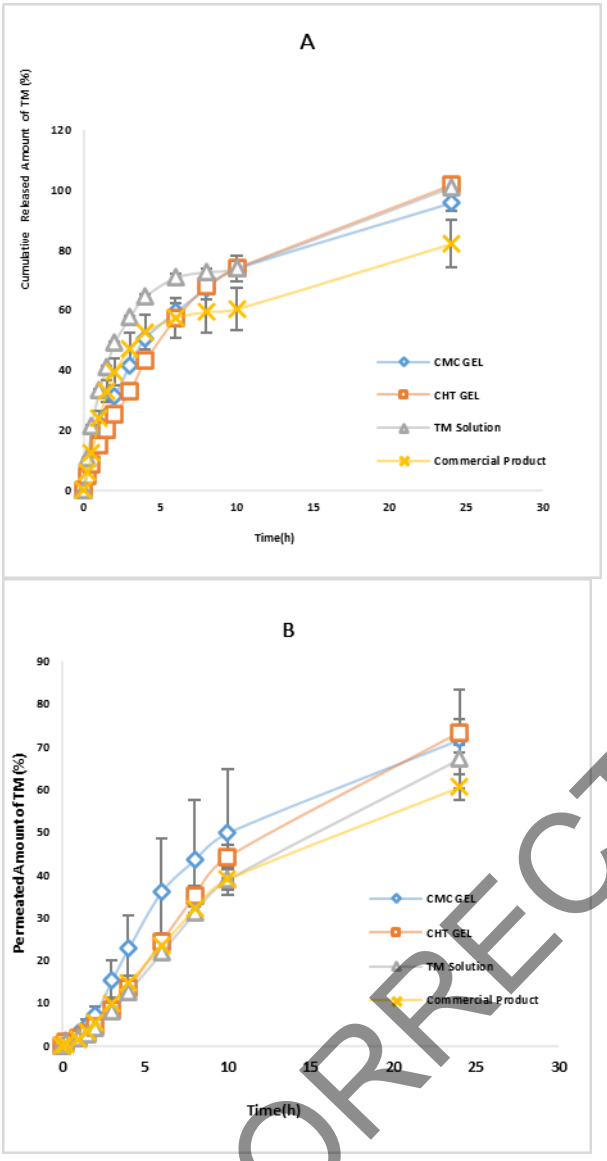

Figure 4. TM release and permeation profile from TM-containing in situ ophthalmic gels through dialysis membranes (A) and cow corneas (B) ($p>0.05$)

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

System	P338:188 (%w/v)	CMC (%w/v)	CHT (%w/v)	Gelation Time (sec)
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

Table 2. The mechanical properties of the gel formulations

	Hardness (g) ±SD	Adhesiveness (g.sec) ± 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