Journal of Research in Pharmacy
Research Article

Design of Metformin HCl and Moxifloxacin HCl Loaded Thermosensitive In Situ Gel

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Received: 29 March 2022 / Revised: 20 June 2022 / Accepted: 20 June 2022

ABSTRACT: Corneal neovascularization (CNV) is a serious ocular surface disease that causes the cornea to lose its transparent structure. It is a difficult disease to treat, so different drugs and different carrier systems are tried in the treatment of the disease. One of these methods is the combined application of metformin HCl (MHL) with a fluoroquinolone group antibiotic. The aim of this study is to load MHL with low permeability into a drug delivery system that can prolong the residence time on the ocular surface. In this context, different in situ gel formulations were produced by using poloxamer, a thermosensitive polymer, together with hydroxypropylmethylcellulose, a natural viscosity increaser. In situ gels were evaluated for clarity, pH, gelation temperature, and rheological behaviors and selected two formulation. After loading MHL and moxifloxacin HCl (MOX) into these two formulations, it was determined that they were clear, had a pH of around 7, a gelling temperature of 34-35 °C, and showed pseudoplastic flow. However, drug loading capacities were found to be over 97%.When in vitro release studies were examined, it was determined that both MHL and MOX released for at least six hours. The results showed that the combination of PF127 and HPMC has potential as an *in situ* gelling systems for ocular delivery of MHL and MOX.

KEYWORDS: Metformin HCl; i*n situ* gel; ocular drug delivery; moxifloxacin HCl; hydroxypropylmethylcellulose

1. INTRODUCTION

Normally, the cornea is a transparent and avascular tissue. However, various infections, inflammations and degeneration of ocular tissues may cause corneal neovascularization (CNV) [1]. CNV is one of the major conditions that cause corneal opacity. It can cause severe vision loss and even blindness in corneal opacity. Therefore, corneal neovascularization is a worldwide public health problem. Angiogenesis is a complex and multistep process. Among them, vascular endothelial growth factor (VEGF) has been proven to be a major regulator of CNV [2]. Anti-VEGF drugs such as bevacizumab and ranibizumab prevent the formation of new blood vessels. These drugs are approved by the Food and Drug Administration (FDA) for the treatment of neovascularization [3].

However, since the eye has a very sensitive and unique structure, it reduces the effectiveness of conventional eye drops. These drugs are rapidly removed from the eye surface, so their bioavailability is below 5%. In order to achieve the desired effect, they may need to be applied in very high doses or in repeated doses, in this case it causes serious toxicity and side effects [4]. Different ocular drug delivery systems have been developed to increase the effectiveness of drugs. One of these systems is in situ gels. In situ gels have been extensively used in ocular applications in recent years. In the preparation of in situ gels, polymers that show sol-gel transition with the change of pH, temperature or specific ions in the environment are preferred. In situ gels prepared in solution or suspension form turn into gel in the area they are applied. In this way, patient compliance and the effectiveness of the drug increase. Studies have demonstrated that corneal residence times of some *in situ* gel systems can be up to a few hours [5,6].

Pluronic (poloxamer) is one of the most preferred polymers for thermosensitive gelling systems. It shows amphiphilic behavior owing to the hydrophilic ethylene oxide area and hydrophobic propylene oxide area. The gelation of poloxamer can be explained by differences in micelle structure depending on the temperature of the environment and the concentration of poloxamer. They are widely

http://dx.doi.org/10.29228/jrp.215
J Res Pharm 2022; 26(5): 1230-1241

used ocularly because they have sufficient inert structure and allow controlled release. It is preferred between 15-25% in ocular applications. However, its viscosity remains low at these doses, and it may have a toxic effect at higher doses. For this reason, different polymers are added in order to increase the viscosity [7]. Hydroxypropylmethylcellulose K4M is one of these polymers (HPMC). HPMC has features that make it ideal for ocular administration. [8].

Metformin HCl (MHL), a typical drug used for the treatment of type 2 diabetes. It work by stimulating the adenosine 5'-monophosphate (AMP)-activated protein kinase and influences the glucose uptake from the blood [9]. It has been observed that Met can be used in the treatment of chronic diseases such as cancer. However, MHL is one of the candidate drugs to prevent neovascularization [10]. A study shows that MHL can inhibit VEGF-induced cell proliferation and exert its anti-angiogenesis effects by suppressing Flk-1 level [11]. However, MHL is a molecule with low permeability despite its high water solubility. When the Biopharmaceutics Classification System is examined, it is seen that it is in class III [12]. For this reason, it is thought that prolonging the contact time with the cornea will have effective results. In another in vivo study, it was determined that MHL was effective in the treatment of CNV [13]. This study was shown that the use of MHL together with an antibiotic increases its effectiveness in the treatment of CNV. For this reason, it was decided to load MHL with Moxifloxacin HCL (MOX) into the drug delivery system to be prepared in our study.

The aim of this study is to prepare in situ gel formulations containing different ratios of PF 127 and different ratios of HPMC. Then, these in situ gel formulations are evaluated in terms of clarity, viscosity, gelation temperature and pH and the appropriate formulation is determined. 0.01% MHL (13) and MOX (%0.5) (MOX's market drug is at this dose) were added to the selected formulation and the loaded in situ gel was evaluated for its in vitro characterization and release properties.

2. RESULTS AND DISCUSSION

2.1. Quantification of metformin HCl and moxifloxacin HCl

Calibration of the method was carried out by measuring a series of standards of diluted stock solutions of MOX and MET. Absorbance values were plotted against MOX and MET concentrations over a range of 0,5-15 ppm.

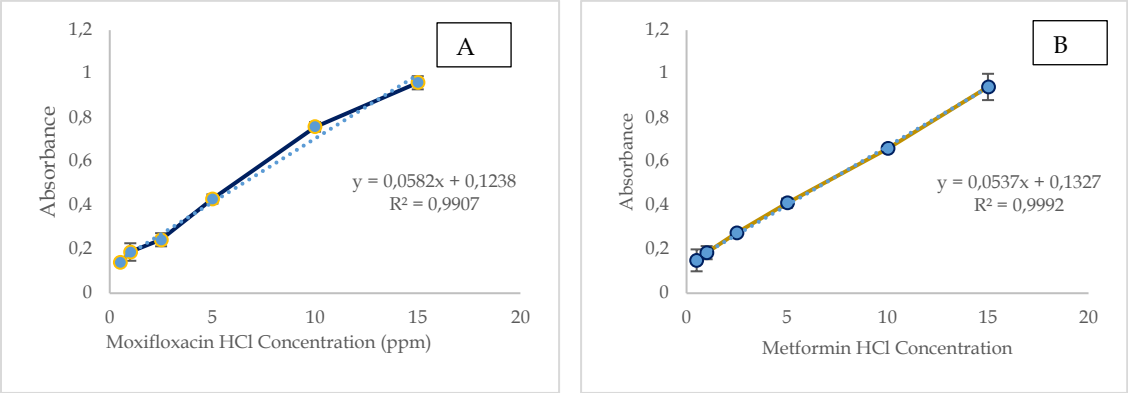


Figure 1. A) MOX calibraion curve with the calibration equation and correlation coefficient B) MOX calibraion curve with the calibration equation and correlation coefficient. The linear regression equation MOX and MET was found as y=0.0582x+0.1238 and y=0.0537x+0.1327 where y is the average and X is concentration (ppm), with a correlation oc 0.09907 and 0.9992, respectively.

2.2. Gelation temperature and clarity

In situ gels should have a low viscosity at room temperature for easy application. In addition, after application to the eye, there should be a sol-gel transition at body temperature (35-36°C) and a transparent structure should be formed. Clarity is one of the critical parameters to increase patient acceptability in situ gels [14]. In this study, different ratios of PF 127 and HPMC aqueous solutions were prepared by determining the appropriate in situ gel formulation to carry MHL and MOX. When the in

situ gel formulations prepared with different ratios of polymers were examined, it was determined that all formulations were clear except for the K15 formulation containing a high amount of 25% PF 127 and 3% HPMC. Other formulations were sufficiently transparent (Table 2).

Gelation temperature is another important parameter for ocular applications. For a successful application, the viscosity should increase in order to increase the contact time with the ocular surface after the application while the viscosity is low at room temperature [15]. When the formulations were examined, it was determined that there was a gelling temperature between 16-37 °C (Table 1). This is due to the polymer concentration. It is seen that the gelation temperature decreases as the amount of PF 127 and HPMC increases. It was determined that the K2 and K4 formulations showed gelation at 35°C-34 °C, respectively. When the literature is examined, it is seen that there are similar results [8].

In aqueous medium, PF127 keeps all molecule seperate at temperatures under the critical micelle temperature (CMT) at which the critical micelle concentration (CMC) happen. When the temperature increases above the CMT, the hydrophilic chains of PF 127 begin to form micelles surrounding the hydrophobic core. In other words, as the PF 127 concentration increases, the CMT value decreases [16].

CMT has a serious effect on the gelling temperature. The main parameter at the gelling temperature is the concentration of the pluronic. As the concentration increases, they form lattice-like structures in water, which causes the gelation temperature to decrease [17]. In our formulations, concentrations of pluronic have been reduced to provide gelation is at corneal temperature. When the literature is examined, it is seen that there are similar results [8].

Table 1: In vitro characterization analysis results of in situ gels

Formulation	pH (±SD)	Gelation temperature (°C±SD)	Viscosity (centipoise) 25 °C	Viscosity (centipoise) 35 °C	Clarity
K1	6.98±0.01	37±0.4	285±15	8852±121	Clear
K2	6.95±0.02	35±0.3	314±35	12878±152	Clear
K3	7.01±0.03	31±0.4	390±47	16645±223	Clear
K4	6.94±0.02	34±0.2	300±29	10402±122	Clear
K5	6.97±0.01	30±0.8	451±36	17548±232	Clear
K6	6.93±0.02	29±0.5	502±45	24722±320	Clear
K7	7.05±0.06	29±0.4	465±31	24842±442	Clear
K8	7.00±0.03	26±0.1	967±65	34742±463	Clear
K9	6.92±0.04	25±0.2	8564±103	38812±524	Clear
K10	7.05±0.02	25±0.3	7565±94	371242±311	Clear
K11	6.91±0.01	22±0.4	11244±321	50152±625	Clear
K12	6.99±0.02	19±0.5	14568±286	52050±526	Clear
K13	6.98±0.03	19±0.7	20085±577	50142±274	Not Clear
K14	7.01±0.04	17±0.3	24085±428	57847±828	Not Clear
K15	6.94±0.10	16±0.8	28985±647	61254±729	Not Clear

2.3. pH value

Since the eye is a sensitive organ, it is seriously affected by pH changes. The pH of the applied formulation is desired to be between 5.0-7.4. In applications outside this range, eye irritation may occur. Furthermore, in applications conducted outside of this range, it promotes tear secretion to balance the pH of the eye, lowering the bioavailability of the ocular application [18,19]. All in situ gel formulations have been found to have a pH of about 7. However, all formulations appear to be at a pH that will not cause ocular irritation.

2.4. Viscosity

Rhelogical behaviors are an important parameter to increase the in vivo usability and effects of in situ gels. Various problems may arise in the application of very high viscous in situ gels, while those with low viscosity are exposed to a rapid elimination from the eye surface with tears. An ocular formulation with a high viscosity is undesirable because it tends to leave a distinct residue on the eyelid side after application. In situ gel formulations with pseudoplastic behavior are suitable for reducing the negative effects of ocular reflexes such as blinking on the formulation. The formulation's pseudoplastic flow allows for a more pleasant application as well as a longer corneal contact time [20].

Figure 2 shows the effect of changes in angular velocities on viscosity. When the rheological behaviors are examined, all in situ gel formulations at their gelling temperatures were demonstraed pseudoplastic flow (shear thinning system) like tear fluid.

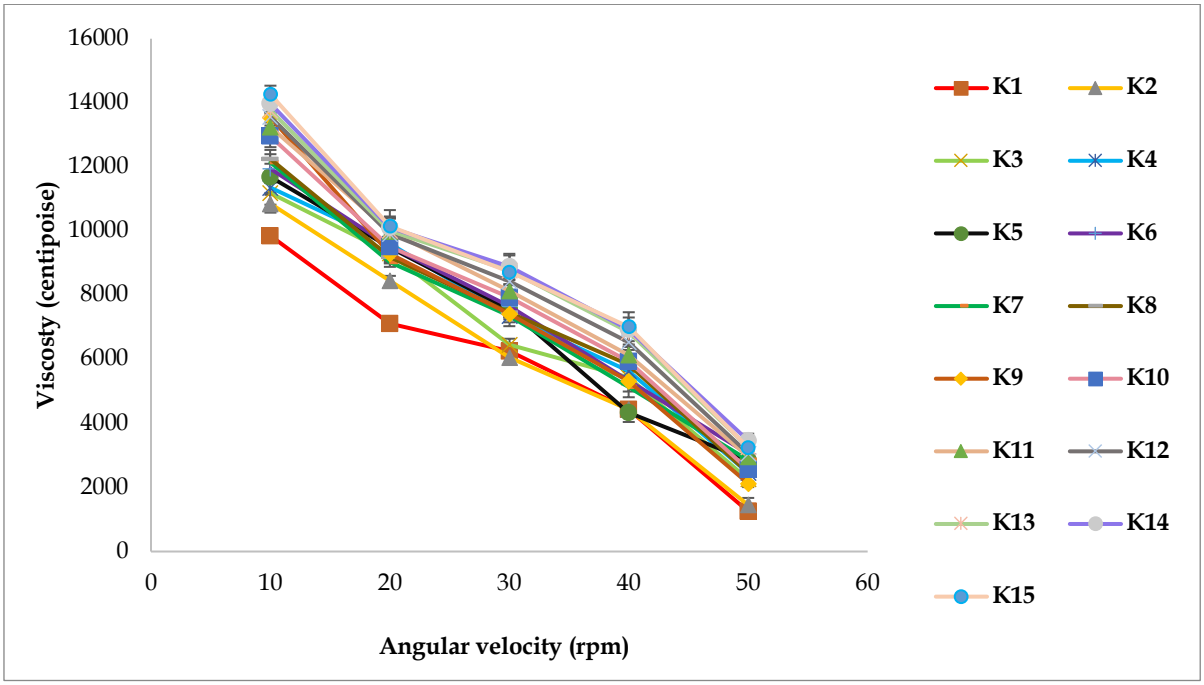


Figure 2. Rhelogical behaviors of in situ gelling systems.

When the literature is examined, it has been seen that the optimum viscosity is between 50–50,000 cp for successful results in ocular application [15]. Therefore, viscosity values were measured for all formulations at 10 rpm at both 25°C and 35°C. When the findings were evaluated, it was discovered that the viscosity values varied depending on the concentrations of PF 127 and HPMC. While the viscosity of the formulation containing 25% PF 127 and 3% HPMC at 25°C was 28985±647 cp, the viscosity of the formulation containing 15% PF 127 and 1% HPMC was 285±15 cp (Table 2). In this case, it shows that polymer concentration has serious effects on viscosity. When the literature was examined, it was determined that there were similar results [15].

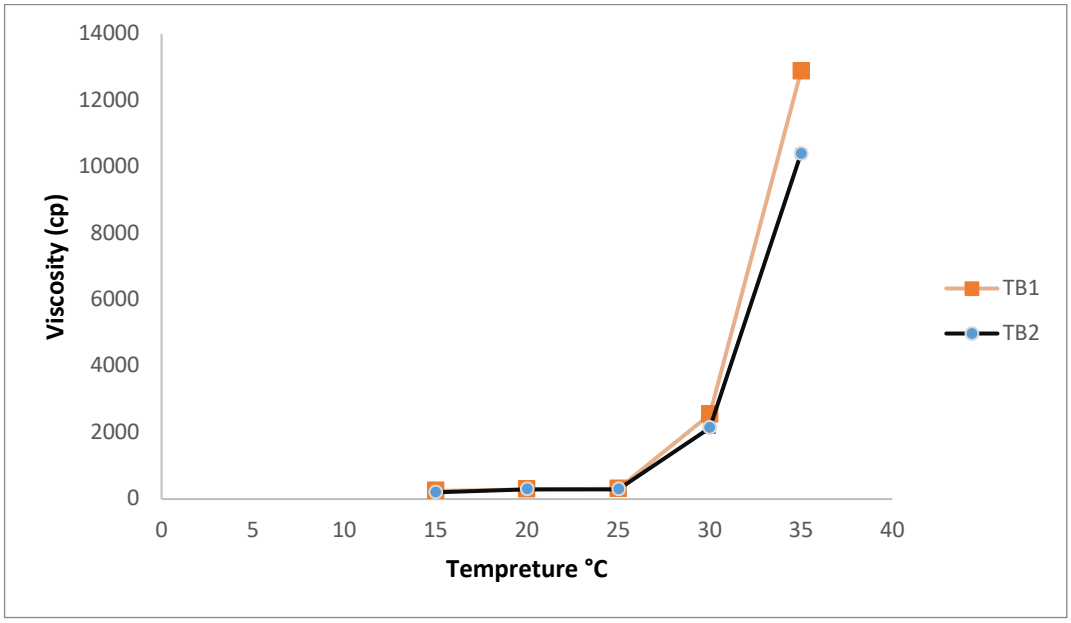


Figure 3: Viscosity profiles of in situ gels at different temperatures.

As a result of the characterization study (pH value, gelation temperature, clarity, and viscosity) on all in situ gel formulations, two formulations were selected. Both MHL (0.01%) and MHL (0.5%) were loaded in the two formulations (Table 3). These formulations are TB1 and TB2. It was determined that TB1 and TB2 prepared using two active substances were clear, their pH values were around 7 and their gelling temperatures were 35°C and 34°C, respectively. In order to evaluate the effect of temperature on the viscosities of the two formulations, their viscosities were measured at 15, 20, 25, 30, 35°C (Figure 3). The viscosity of both formulations increased dramatically as the temperature rose. When looking at the literature, it's been discovered that the viscosity of a substance increases dramatically as the temperature rises [21].

Table 2: Physical properties of drug containing formulations and their component.

Formulation components and physical properties	TB1	TB2
MHL (% w/v)	0.01	0.01
MOX (% w/v)	0.5	0.5
PF 127	15	15
HPMC 4K	10	10
BAK	0.001	0.001
EDTA	0.05	0.05
Sodium chloride	0.2	0.2
Distilled Water	100 ml	100 ml
pH (±SD)	6.97±0.01	6.96±0.02
Gelation temperature (° C±SD)	35±0.3	34±0.2
Viscosity (centipoise) 25 °C	314±35	300±29
Viscosity (centipoise) 35 °C	12878±152	10402±122
Clarity	Clear	Clear

2.5. Drug loading

It has been determined that the loading capacity of TB1 and TB2 both MHL and MOX is over 97% (Figure 4). This shows that polymer concentration has no effect on drug loading. When the literature is examined, it is seen that there are similar results.

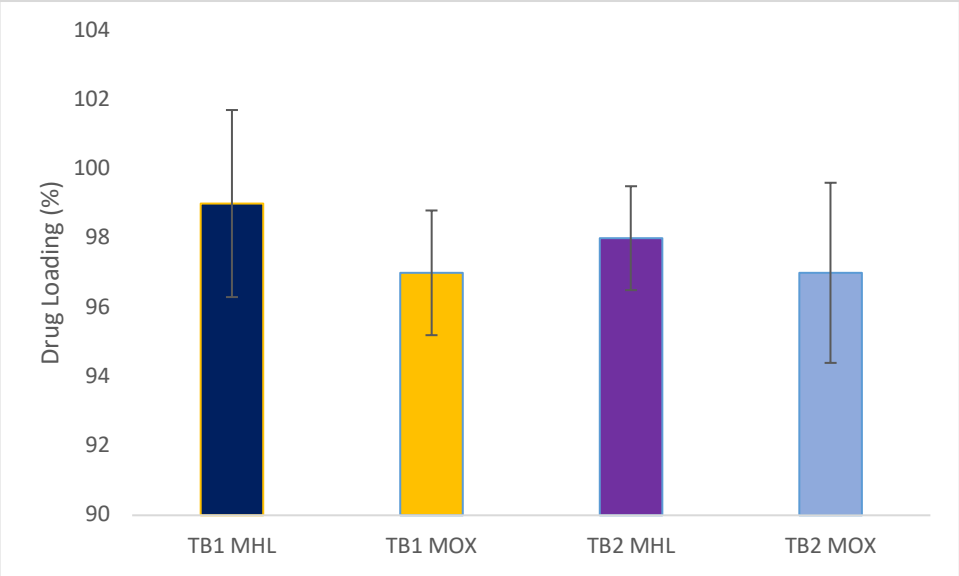
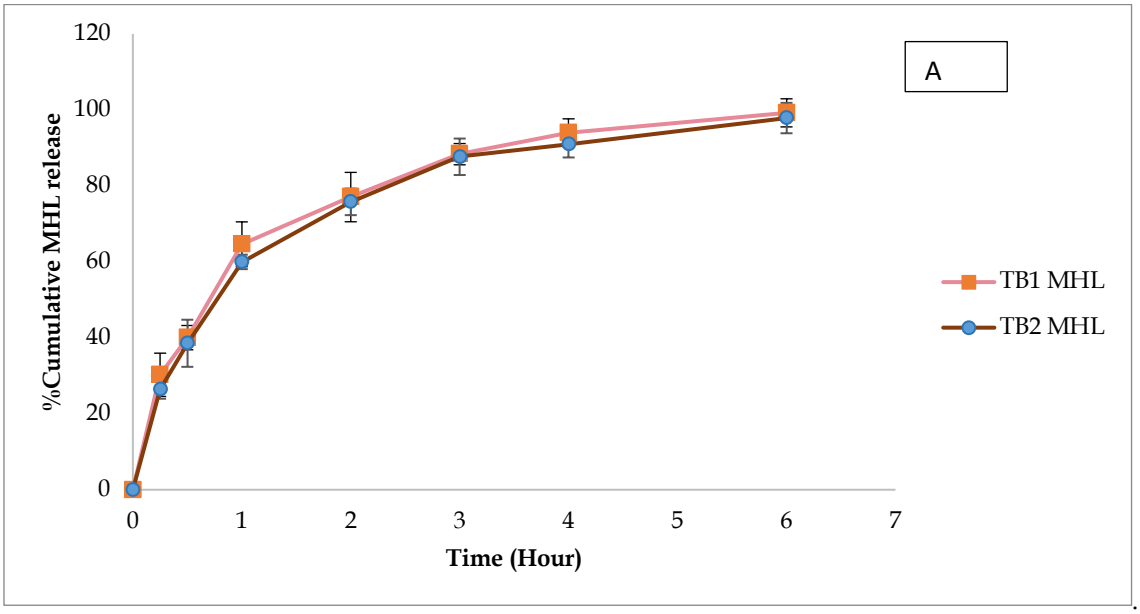


Figure 4: Drug loading given in percentages for MHL and MOX (n=3)

2.6. Drug release

MHL (0.01 %w/v) and MOX (0.5 %w/v) loaded formulations were used, and in vitro drug release of in situ gels was performed at 35°C in isotonic phosphate buffer pH 7.4. In Figure 5, A and B show the in vitro release profiles of MHL and MOX, respectively. When the release profiles of TB1 and TB2 for MHL are examined, two formulation showed >65% drug release after two hours, and at the end of 3 hours, 75% of the MHL was released. However, when the release profiles of TB1 and TB2 for MOX are examined two formulation showed >54% drug release after two hours, and at the end of 3 hours, 65% of the MOX was released. At the end of the sixth hour, while the MOX had released 90%, MHL had finished its drug release. As a result, the sixth hour was regarded as the final medication release time. The time when the entire MOX ends is the 7th hour.

This difference in release is thought to be due to the difference in solubility of the two drugs. MHL has a faster release than MOX because of its increased water solubility. When the literature was examined, it was seen that the higher the solubility, the higher the release rate of the active substance. In one study, levofloxacin and metronidazole were loaded on in situ gels, later in vitro release studies showed that levofloxacin HCl released in a faster time due to its higher solubility [22]. In another study by Polat et al., the release properties of besifloxacin HCl and besifloxacin HCl / cyclodextrine complex were compared, and the release time was reduced from seven days to three days as the solubility increased with the complex formed [19].



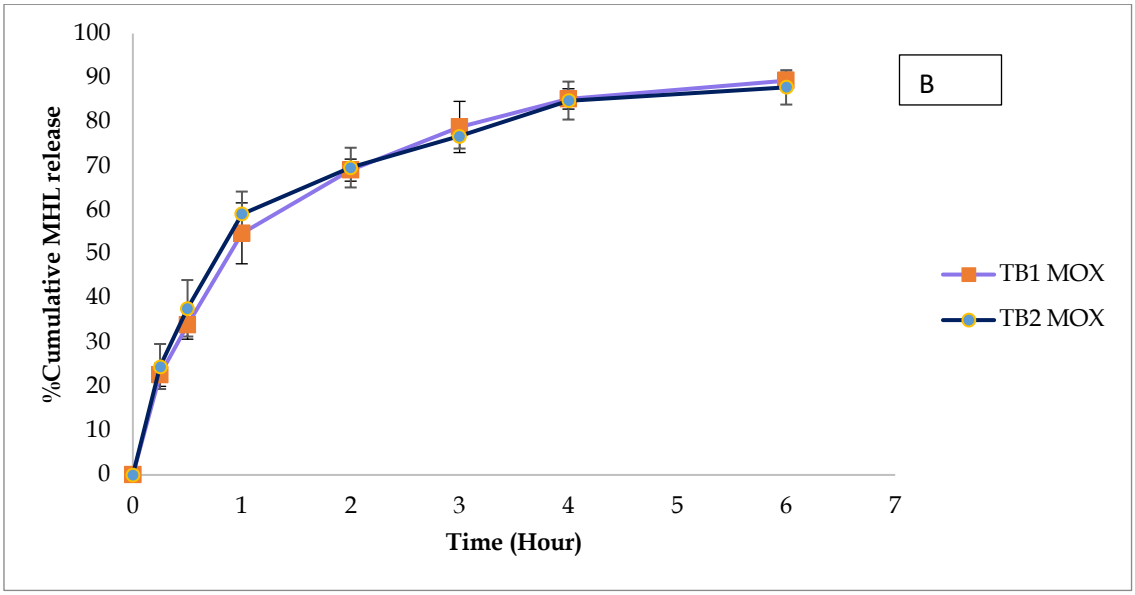


Figure 5. A-B cumulative release of MHL and MOX from in situ gel

The analysis and modeling of drug releases become complicated with polymer in formulation involved. Water-swellable polymers like poloxamer and HPMC are used to make in situ gels. These polymers release by diffusion or erosion-controlled mechanism or a combination of both [23]. The drug release kinetics of MHL and MOX from in situ gel was calculated by using non-linear regression model of KinetDS. The parameters and determination coefficients (R2) calculated with this method are indicated in Table 3. In the study, evaluation was made according to different models such as zero-order, first-order, Hixson–Crowell, Higuchi, Weibull and Korsmeyer–Peppas models. When the regression coefficients are evaluated, the release kinetics for the two active substances in TB1 and TB2 is the model defined by Weibull. In literature, when some studies in the literature are examined, it indicated that the release data from swellable polymeric systems fits best with the Weibull model [24-26].

Table 3: In-vitro release kinetic parameters of MHL and MOX from in situ gel

	Sample	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Korsmeyer-Peppas (R^2)	Weibull (R^2)	Hickson-Crowell (R^2)
	MHL	0.8033	0.6990	0.7105	0.9608	0.9837	0.7352
TB1	MOX	0.8050	0.6848	0.8387	0.9589	0.9927	0.7276
	MHL	0.8068	0.6927	0.7999	0.9626	0.9959	0.7332
TB2	MOX	0.7802	0.6589	0.6975	0.9428	0.9832	0.7014

3. CONCLUSION

Various polymer solutions containing various ratios of PF 127 and HPMC were created as part of the research. In vitro characterization (pH, clarity, gelation temperatures, and rheological behavior) of all these formulations was performed. All formulations were found to have a tear-like pseudoplastic flow. When the gelling temperatures were examined, it was seen that the gelation temperature decreased as the amount of poloxmaer increased. For this reason, K2 and K4 formulations, which gel at eye temperature, were chosen. However, the pH of all formulations was found to be around 7. Since the ocular application range is between 5.0-7.4, it has been concluded that the formulations will not cause irritation due to pH. Then, two formulation were loaded wit MHL and MOX. The pH of these two formulations was 7.0, the gelation temperature was 34-35°C, and pseudoplastic behavior was observed. In the viscosity studies carried out at increasing temperatures for the two formulations, it was observed that the viscosity increased significantly with the increase in temperature. Moreover, it was determined that the drug loading capacity of the two formulations was over 97%. However, since MHL has a higher solubility, it has been observed that Mox, which is released for 6 hours, is released close to 90% in the same period. It is thought that increasing the contact of drugs with low permeability such as MHL with the ocular surface will increase their bioavailability. However, it is thought that co-administration with an antibiotic derivative will increase the success in the treatment of CNV. The medicine's residence duration on the eye was extended with these two formulations, and the drug was released for a longer period of time than with traditional eye drops. Therefore, the two formulations are considered promising drug delivery systems for MHL and MOX.

4. MATERIALS AND METHODS

4.1. Materials

Metformin (MHL), moxifloxacin HCL (MOX), pluronic F-127 (PF 127), hydroxypropylmethylcellulose K4M (HPMC), benzalkonium chloride (BAK), disodium EDTA, sodium chloride and phosphate buffered saline (PBS) tabletwere purchased from Sigma, Steinheim, Germany.

4.2. Production of in situ gel

In situ gels were prepared by the cold method [27]. In this method, PF 127 (%15,17.5,20,22.5 and 25) and HPMC (%1,2 and 3) were dispersed in distilled water and then mixed for 1 hour and kept in the refrigerator at 4°C overnight. One day later, HPMC solution was added onto the PF 127 solution and stirred for 1 hour and stored at 4°C overnight. Then, benzalkonium chloride (%0.001), disodium EDTA (%0.05), and sodium chloride (%0.2) were added to the solution mixture. Each example was stored at 4°C before use. The finally in situ gel formulations are shown in Table 4.

Table 4: Components of in situ gels

	Content of ingredients in each formulation (%, w/w)					
Formulation	**PF127**	**HPMC K4M**	**BAK**	**EDTA**	**Sodium chloride**	**Distile water (qs)**
K1	15	1	0.001	0.05	0.2	100
K2	15	2	0.001	0.05	0.2	100
K3	15	3	0.001	0.05	0.2	100
K4	17.5	1	0.001	0.05	0.2	100
K5	17.5	2	0.001	0.05	0.2	100
K6	17.5	3	0.001	0.05	0.2	100
K7	20	1	0.001	0.05	0.2	100
K8	20	2	0.001	0.05	0.2	100
K9	20	3	0.001	0.05	0.2	100
K10	22.5	1	0.001	0.05	0.2	100
K11	22.5	2	0.001	0.05	0.2	100
K12	22.5	3	0.001	0.05	0.2	100
K13	25	1	0.001	0.05	0.2	100
K14	25	2	0.001	0.05	0.2	100
K15	25	3	0.001	0.05	0.2	100

4.3. Characterization of *in situ* gel formulations

Different concentrations of PF 127 and HPMC (formulation codes K1–K15) were determined for gelation temperature, pH, clarity, and viscosity. Results are indicated in Table 2.

4.3.1. pH

The pH of the gels in situ was measured using a pH meter (HANNA, Germany). The analyzes were done three times (n=3).

4.3.2. Clarity

The clarity of the in situ gels were examined under intense light on a black background after gelation [28] (Table 2).

4.3.3. Gelation temperature

All polymer solutions were heated to 1°C/min at 100 rpm. The temperature at which the magnetic bar stopped moving was marked as the gelling temperature. (Thermomac-TM19). The analyzes were done three times (n=3).

4.3.4. Viscosity

Viscosities of all formulations at both 25°C and 35°C were measured with a fungilab viscometer (USA) with R5 spindle at 10 rpm (Table 2). The analyzes were done three times (n=3).

4.3.5. Rheological studies

The rheological studies of *in situ* gels were done by fungilab viscometer (USA) with R5 spindle. The spindle runs at 1, 2.5, 5, 10, 20, 50 rpm angular velocity. Viscosities of in situ gels were measured at their gelation temperature. Viscosities were measured at different angular velocities, and flow curves were determined. The analyzes were done three times (n=3).

4.4. Production of MHL and MOX contained ın situ gel formulation

In situ gel formulations were evaluated in terms of pH, viscosity, clarity and gelling temperature and suitable formulations were determined. MHL (0.01%) and MOX (%0.5) was added to the two selected formulations. These two formulations were named TB1 and TB2. The formulation components in TB1 and TB2 and the characterization results of these formulations are shown in Table 2.

4.5. Drug content

To determine the amount of MHL in in situ gel, 1 mL of in situ gel was diluted in 2 mL of water., then the solution was sonicated for 1 h in a bath sonicator MHL and MOX concentrations were determined by UV–vis spectrophotometer (UVmini-1240 Shimadzu Japan) at 233 nm and 299 nm, respectively [29].

$$\text{Drug loading (\%)}=\frac{Amount\ to\ encapsulated\ MHL}{Total\ weighted}x100$$

4.6. In vitro release studies

Dialysis bag method is frequently used in the release studies of in situ gels [30]. The formulation containing MHL (100 µL) was transferred to the dialysis bag, then the dialysis bag was tightly closed and placed in 15 mL of pH 7.4 isotonic phosphate buffer at 37 °C. Since MHL has a high water solubility. the sink condition is provided for 5 mL. At different times (15, 30, 60, 120, 180, 240, 360 min) all 5 mL of the medium was taken and fresh buffer was added instead of the sample. MHL and MOX concentrations were determined by UV–vis spectrophotometer (UVmini-1240 Shimadzu Japan) at 233 nm and 299 nm, respectively. In this way, the amount of release of MHL in different time periods was calculated. The analyzes were done three times (n=3).

Acknowledgements: This study was not supported financially.

Author contributions: Concept – K.P; Design – K.P.; Supervision – K.P.; Resources – K.P.; Materials – K.P.; Data Collection and/or Processing – K.P.; Analysis and/or Interpretation – K.P.; Literature Search – K.P.; Writing – K.P.; Critical Reviews – K.P.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript

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