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Original Article

# Flurbiprofen-encapsulated microsphere laden into heat-triggered *situ* gel for ocular delivery

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## ABSTRACT

**Background and Aims:** This investigation aimed to enhance flurbiprofen's (FB) pre-corneal residence period and ocular availability in the postoperative management of ocular inflammation.

**Methods:** The microsphere (MS) material was poly (lactic-co-glycolic acid) PLGA, and FB-laden microspheres were prepared using a single emulsification/solvent evaporation process, loaded onto thermosensitive in situ gels, and then characterised.

**Results:** The size of the microparticles was measured as 19.3±2.1 µm. Entrapment efficiency, zeta potential, and *in vitro* release; it was observed that the microspheres were released for six days. The optimum gelling capacity was obtained using 15% Poloxamer 407, 8% Poloxamer 188, and 1% HEC (viscosity 80-125 cp). Poloxamer 407 concentration was reduced, resulting in increased gelation temperature and duration. It was determined that the gelling temperature of the selected formulation was 35±0.1 °C, the pH was 6.9±0.02, and the viscosity at the gelling temperature was 11042±247 cP. The addition of hydroxyethyl cellulose increased the mucoadhesive strength. - in vitro release of FB from FB-PLGA MS followed the Korsmeyer-Peppas model , –, and the in situ gel preparation was found to be compatible with the Peppas-Sahlin model. In addition, MTT analysis of the ARPE-19 cell line revealed that the in situ gel formulation was biocompatible.

**Conclusion:** Flurbiprofen release was sustained, ocular availability was improved, and residence time was increased when flurbiprofen-loaded microspheres were incorporated into in situ gel bases.

**Keywords:** Flurbiprofen, Poloxamer Gel, Ocular Gel, PLGA Microsphere, Release Kinetics

## INTRODUCTION

Flurbiprofen (FB) has recently been used to reduce pain and inflammation during ocular surgery; eye drops are the most common type of market FB preparations for ocular administration. However, ocular drops have several drawbacks, such as rapid tear turnover. Numerous experiments have been conducted to enhance the ocular bioavailability of FB (Anderson & Shive, 1997).

A technique used to increase the bioavailability of hydrophobic medicines is the development of PLGA microspheres. To prevent the drug from being metabolised by enzymatic processes in the tear film and to provide consistent and extended drug release, the medication is completely integrated into the polymer matrix (Samatı, Yüksel, & Tarımcı, 2006).

The development of in-situ gel when in contact with the ocular surface as a result of the action of pH, salts, or temperature is the subject of the most current study to address the problem may address the drawbacks of commercial eye drops (Khan, Warade, & Singhavi, 2018).

An optimised formulation was developed using poloxamer 188 (P188) and poloxamer 407 (P407) with FB-loaded PLGA MSs, which forms gelat the corneal temperature, to enhance its bioavailability in ocular tissues. In this study, FB-loaded MC was produced, and the zeta potential, polydispersity index, and particle size were used to describe MSs. UV spectrophotometry was used to determine the effectiveness of FB encapsulation (Samatı et al., 2006). Later, FB-loaded microspheres were included into an optimised in situ gel formulations, which were created to extend the residence period of the microspheres on the ocular surface. The addition of HEC to poloxamer at various concentrations improved bioadhesion properties of the gel the . Then, the optimum formulation was selected, release research was conducted using this formulation, and *in vitro* characterisation experiments of the produced in situ gels were performed.

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Release and kinetic studies were performed to evaluate the selected optimum gel formulation.

## MATERIALS AND METHODS

### Materials

From Sigma-Aldrich (St. Louis, MO, USA), we obtained flurbiprofen, PLGA, poloxamer 407, poloxamer 188, hydroxyethyl cellulose (HEC) (125 cp), Benzalkonium chloride (BAC), NaCl, dichloromethane, PVA, and phosphate buffered saline (PBS) tablets.

### Production of Flurbiprofen-loaded PLGA microspheres

FB-loaded PLGA MCs were produced using a slightly modified single emulsification/solvent evaporation technique (O/W) (Momoh, Adedokun, Lawal, & Ubochi, 2014). Briefly, 400 mg of PLGA and 200 mg of FB were dissolved in 5 mL of dichloromethane (DCM) to create the organic phase. The O/W emulsion was created by emulsifying the organic phase into an aqueous phase (30 mL) prepared with PVA as a dispersion agent in water (3% w/v) and homogenised (9000 rpm for 4 min) with Ultra-Turrax (IKA, Germany). Following the homogenisation step that created the emulsion, 20 ml of distilled water was added, and mixing was carried out at the same speed for the next minute. The immature microspheres were agitated for 5 h at 500 rpm while suspended in 100 mL of distilled water to produce a completely O/W emulsion. The organic solvent removed throughout the night by stirring. (RCT Simple-Ika). The microspheres separated with centrifugation at 5000 rpm for 10 min in a Thermo ScientificTM SL 16R. They were dried overnight at room temperature following three washings with deionised water. The dried microspheres were stored in a desiccator for further examination. The same method was applied to create PLGA microspheres that were blank and did not contain FB. Figure 1 shows a schematic of microsphere production.

### Spectrophotometric Analysis of Flurbiprofen

Using a Shimadzu UV-1800 Spectrophotometer (Japan), the amount of FB in vitro was measured spectrophotometrically at 246 nm, as previously described (Akyüz, Duman, & Murat, 2017).

### *In vitro* characterisation of Flurbiprofen-loaded PLGA Microspheres

*Particle size and zeta potential:* The distributions of particle size, mean zeta potential, and particle size of the produced microspheres were calculated using Mastersizer 2000 and the light scattering principle (Malvern corporation, UK). Each measurement was performed three times.

*Surface morphology of FB-loaded PLGA MSs:* The morphological properties and surface traits of PLGA MSs were observed by scanning electron microscopy (SEM) (Zeiss Evo LS-10, Germany).

*Determination of drug loading capacity:* To determine how well the microspheres were entrapped, perfectly measured (20 mg) FB-loaded PLGA microspheres were dissolved in 1 mL of DCM and then transferred to 9 mL of methanol. The suspension was centrifuged, and the supernatant was then filtered through a 0.45-m membrane. The supernatant was examined using a UV/visible spectrophotometer set at 246 nm. A calibration curve was created using previously known standard solutions of FB in DCM/methanol mixtures (1:9). For each batch, measurements were performed in triplicate. The spectrophotometric method was validated following the recommendations of the ICH. Within the scope of the validation of the spectrophotometric method used for FB quantification, linearity, accuracy, precision, repeatability, limit of detection (LOD), and limit of quantification (LOQ) were determined. The regression coefficient ($r^2$), which expresses linearity, was determined for FB as $r^2 = 0.9898$. The LOD and LOQ, which express the sensitivity of the method, were determined as 0.0217 µg/mL and 0.0719 µg/mL, respectively. The following equation is used to compute the encapsulation efficiency:

$$EE\ \% = (actual\ drug\ content / theoretical\ drug\ content) \times 100 \quad (1)$$

*In Vitro release of FB from FB Loaded PLGA MCs*. The collection and separation approach, which is often employed for polymeric microparticles, was used to conduct in vitro release tests. Evaluation of FB release from PLGA MCs was carried out under sink conditions based on FB solubility (5 g/mL) (D'Souza & DeLuca, 2006). Briefly, 40 mL of PBS (pH 7.4) containing 10 mg of microspheres was agitated horizontally at 30 rpm for six days at 37 ºC. The samples taken at certain time intervals were analysed spectrophotometrically (0, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, and 144 hours). Sample replacement was performed using the same amount of new-release medium (Samdancioglu, Calış, Sumnu, & Atilla Hincal, 2006). It was feasible to determine the cumulative proportion of total FB emitted at each time point using the previously disclosed confirmed spectrophotometric approach.

### Release kinetics

DDSolver, a programme developed to speed up calculations and avoid computational mistakes, was used to investigate the release profiles of microspheres and microspheres embedded in in situ gel formulations. Data were computed using the DDSolver programme after acquiring the *in vitro* release profiles of microspheres to identify the most vital and significant four criteria; coefficient of determination (R2), adjusted coefficient of determination (R2adjusted), Akaike Information Criterion (AIC), and model selection criteria (MSC). The resulting data were fitted to multiple kinetic models (Zero order, First order, Hixson-

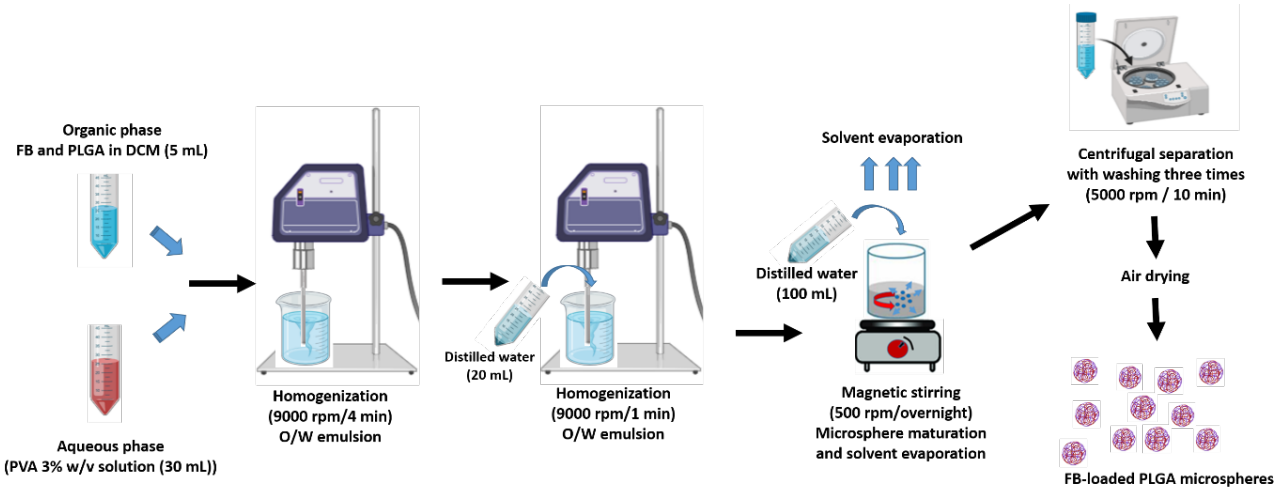


**Figure 1.** The method for producing FB-loaded PLGA MC.

Crowell, Higuchi, Weibull, Korsmeyer-Peppas, Peppas-Sahlin, and Hopfenberg model) (Zhang et al., 2010). The highest $R^2$, $R^2_{adjusted}$, MSC, and AIC values were used to evaluate the different kinetic models. Additionally, DDSolver was used to calculate the release differences between AS-free and FB-loaded PLGA MSs using the "difference (f1)" and "similarity (f2)" factors (Puthli & Vavia, 2009).

### *In situ* gel preparation

Using the cold technique, in situ gels were prepared (H Kerem POLAT, 2022). Other PX 407 and PX 188 concentrations with mucoadhesive HECs were used to produce FB-loaded MS-in situ-forming gels. The components of the ready formulations are listed in Table 1. For the formulations, the projected amount of cold water in a vial with a magnetic bar was gradually increased for PX 407 and PX 188. The mixture was continually stirred with a magnetic stirrer (Thermomac-TM19). The Pluronic solutions were partially dissolved and occasionally blended to create clear homogeneous solutions. Additional amounts of the mucoadhesive HEC polymers (0, 0.5, and 1%) (wt/wt) were added to the total poloxamer content during preparation (Table 1). Each sample was stored at 4°C before use. In all formulations, Benzalkonium chloride (BAC), NaCl, and FB-loaded MC (%0.03) were added.

### *In vitro* characterisation of *in situ* gels

*pH:* The pH was determined using a pH metre (Fungilab viscometer (USA). Three measurements were taken (n=3). For ocular formulations, isotonicity is a crucial factor to consider preventing ocular irritation and tissue damage. The test was conducted using the hemolytic technique (H Kerem POLAT, 2022)

*Clarity:* Following gelation, the in situ gel formulation's clarity was assessed by holding it up to bright light while contrasting it with a dark background (Polat, 2022).

*Gelation Temperature:* Each polymer solution (10 ml) was stirred with a magnetic stirrer in a water bath. The heated polymer solutions were swirled at 100 rpm and 1 °C/min (Thermomac-TM19). The temperature at which the magnetic bar stopped moving was recorded as the gelling temperature. Each sample was subjected to three measurements. (Polat, 2022).

*Determination of gelation time:* A test tube containing 2 ml of sample kept at 4 °C was placed in a water bath heated to the gelation temperature (35 °C). By frequently turning the test tube upside down, the in situ gel was checked for gelation. In cases in which the test tube did not flow when turned upside down, the gelation time was recorded (Asasutjarit, Thanasanchokpibull, Fuongfuchat, & Veeranondha, 2011).

**Table 1.** Compositions of *in situ* gelling formulations

| Code | Formulation's ingredients (%, w/w) | | | | | | |
|---|---|---|---|---|---|---|---|
| | FB-loaded MCs | PX 407 | PX 188 | HEC (80-125 cP) | Benzalkonium chloride (BAC) | NaCl | Water |
| A1 | 0.03 | 12 | 8 | 0 | 0.001 | 0.2 | 100 |
| A2 | 0.03 | 12 | 8 | 1 | 0.001 | 0.2 | 100 |
| A3 | 0.03 | 12 | 8 | 1.5 | 0.001 | 0.2 | 100 |
| A4 | 0.03 | 15 | 8 | 0 | 0.001 | 0.2 | 100 |
| A5 | 0.03 | 15 | 8 | 1 | 0.001 | 0.2 | 100 |
| A6 | 0.03 | 15 | 8 | 1.5 | 0.001 | 0.2 | 100 |
| A7 | 0.03 | 18 | 8 | 0 | 0.001 | 0.2 | 100 |
| A8 | 0.03 | 18 | 8 | 1 | 0.001 | 0.2 | 100 |
| A9 | 0.03 | 18 | 8 | 1.5 | 0.001 | 0.2 | 100 |

*Excipient ratios were kept constant throughout the study.

*Viscosity:* The viscosities of the samples were evaluated using a Fungilab rotating viscometer (USA) with an R5 spindle at 10 rpm. At 25 and 35 °C, the viscosity values of all MS-loaded in situ gels were measured (Table 2) (Polat, 2022).

*Rheological behavious:* The rheological behaviours of the samples were measured using a Fungilab rotating viscometer (USA) with an R5 spindle at different (1, 2.5, 5, 10, 20, and 50) rpm. Viscosities were evaluated at various angular velocities, and flow curves were computed.

**Determination of the Mucoadhesive Force**

The *in vitro* bioadhesive force on sheep cornea tissue obtained locally from butchers was measured using a piece of equipment known as a texture analyser (TA.XT Plus, StableMicro Systems, UK). Bioadhesion studies were conducted on all formulations. The in situ gel was applied to one of the texture analyser's arms, and the eye was placed on the others. The probe used to apply the membrane to the basis decreased at a force of 0.2 N and a speed of 0.1 mm/s during the 60-s contact period. The effectiveness of the in situ gel bioadhesive was evaluated by testing resistance to probe removal ( Polat et al., 2023). At 35 °C, triplicate measurements of each bioadhesion were performed.

***In vitro* Release of FB from FB-MC–loaded in situ gels**

The in vitro release study was conducted using the dialysis bag method (Polat et al., 2022). The dialysis membrane (Sigma, D9277) was then sealed, 100 µL of MHM was added, and 10 mL of an isotonic phosphate buffer (pH 7.4) was placed at 35 °C. This describes the sink condition is provided. All media were removed at different time points, and 10 mL of the new buffer media was introduced (0, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, and 144 hours). The amount of FB was measured using a UV-Vis spectrophotometer.

**Biocompatibility studies (MTT assay)**

A 37°C humidified 5% CO2 incubator was used to culture ARPE-19 cells in DMEM/F-12 medium containing 10% foetal bovine serum, 50 unit/mL penicillin, and 50 mg/mL streptomycin. The MTT assay was used to assess how the inserted formulations affected the viability of ARPE-19 cells. After counting the cells using the Thoma slide counting chamber, a 96-well plate was seeded with 5000 cells per well at a density of 50000 cells/ml. The contents of the wells were removed overnight. FB-loaded mc-containing in situ gels were used to treat cells at dosages of 5, 10, 15, 20, and 25 µg/ml. (Aksungur et al., 2011; -Polat et al. (2023). DMEM was used to manufacture blank in situ gel stock solutions, and complete medium was used to make dilutions. After injecting the medication and in situ gel, the cells were cultured for 2 h. Then, 25 µl of MTT solution (5 mg/ml) was added to each well, and the cells were incubated for 24 h. Following the addition of 200 µl of DMSO to each well, absorbance was measured using an Elisa Plate Reader.

***Ex vivo* Corneal Transport Studies**

*Ex vivo* corneal transport investigations were performed using locally obtained sheep corneal tissue from butchers. For evaluating the passage and participation of FBs through ocular tissues, the formulations were compared with commercial preparations. Transport studies were conducted with n = 3. Two distinct formulations were examined, one of which could be purchased (Flubord®). The "vertical diffusion Franz cells" -system was used in transport studies.

The donor side of the tissue transport cells was towards the surface of the outer cornea, and its contact area was 0.785 $cm^2$. The donor side received 2 mL of the in situ gel formulation and commercial product, whereas the acceptor side received 4 mL of PBS (pH 7.4) as the buffer. Without the use of a stirring element, the system was maintained at 34°C in a light-proof container. After 2 h, samples were collected from the recipient cell into vials, and a UV-vis spectrophotometer was used to measure the FB drug loading capacity.

### *Statistical analysis*

Using unpaired one-way ANOVA and Tukey's multiple comparisons test, statistical significance was determined. (labelled as * = P < 0.05, ** = P < 0.01, *** = P < 0.001, **** = P < 0.0001 and ns = not significant.

## RESULTS

### *In vitro* characterisation of FB-loaded PLGA MCs

Table 2 shows the average zeta potentials, encapsulation efficiencies, particle sizes, and distributions of FB-loaded and blank PLGA MCs. The average particle size of FB-loaded microspheres was 19.3 µm. The average particle size of blank microspheres was discovered to be 24.1 µm.

Figure 2 shows a SEM image of the shapes of FB-loaded and -unloaded PLGA microspheres. The microspheres were mostly spherical in shape and had tiny sporadic pits all over their surfaces. Surface holes were more noticeable in microspheres loaded with FB. Moreover, greater magnifications allowed for a more detailed observation of the microsphere surfaces, and the particle size distribution in the photographs matched the results of the mastersinger device (Figure 3).

*The in vitro* release of FB from PLGA MCs was continued over a period of six days in accordance with the extended-release goal of the study, as illustrated in Figure 4. According to previous studies, two- or three-phase release profiles can be observed when drugs are released from biodegradable polymers through both diffusion and erosion of the polymeric matrix. The initial and immediate release of the burst effect continued for eight hours. After 72 hours, a second rapid release phase appeared. Subsequently, a continuous release profile was observed through day 6 of the experiment.

### *In vitro* Gel Characterisation

To find the best compositions for use as in situ gels, systems with various concentrations of PX188 and PX407 and different concentrations of HEC were created and examined for gelling capacity. Table 3 presents the gelling properties of the formulations.

The lowest polymer-containing samples, A1, A2, and A3 (13% PX407, 8% PX188), demonstrated a sol (liquid) state under non-physiological (4°C) and physiological (35°C) conditions with no gelation, indicating that they could not gel.

The pH of the FB-loaded MS-loaded in situ gel compositions was measured using a pH meter that had already been calibrated. The pH of the in situ gel compositions was found to range between 6.78±0.04 and 7.02 ± 0.01 (Table 3).

### Rheology and viscosity

Figure 5 shows the viscosity measurements of FB-loaded MSs in situ gelling systems at different shear rates. At the moment of gelation, all the produced formulations exhibited shear-thinning flow characteristics.

This study investigated the changes in viscosity with increasing temperature (Figure 6).

### Determination of the Mucoadhesive Force

The mucoadhesive force, which extends the formulation's precorneal residence time by preventing quick drainage, is a crucial physicochemical characteristic for the in situ production of ophthalmic gels. Figure 7 presents the analysis of the mucoadhesive forces of all the created in situ gels.

### *In Vitro Release of FB from FB-MC-loaded In Situ Gels*

Experiments on the in vitro release of medication were performed using in situ gels at 35 °C in an isotonic phosphate buffer (pH 7.4) and MHM (0.03%). The in situ gels loaded with MHM (0.03%) and the MSs loaded with FB underwent 144 h of *in vitro* release profile analysis (Fig 4).

### Biocompatibility studies (MTT assay)

Drug delivery vehicles should be biocompatible as novel ocular drug delivery systems. Figure 8 shows the biocompatibility of the in situ gel.

### *Ex vivo* Transport Studies

The *ex vivo* transport study results (Fig. 9) showed that MHM provided an enhanced drug concentration compared with the marketed product, Flubord (P < 0.01).

**Table 2.** Results of FB-loaded and blank PLGA MCs' *in vitro* characterisation (n=3)

| **Blank PLGA MCs** | | | | | **FB-loaded PLGA MCs** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Particle size (µm)** | | | **Zeta Potential (mV)** | **Polydispersity Index (PDI)** | **Particle size (µm)** | | | **Zeta Potential (mV)** | **Polydispersity Index (PDI)** | **Entrapment Efficiency (%)** |
| $D_{10\%}$ | *Mean Particle Size* $D_{50\%}$ | $D_{90\%}$ | | | $D_{10\%}$ | *Mean Particle Size* $D_{50\%}$ | $D_{90\%}$ | | | |
| 2.2± 0.4 | **19.3 ± 2.1** | 37.4 ± 2.7 | -33.6± 2.1 | 0.316 ± 0.011 | 3.8 ± 1.3 | **24.1± 4.6** | 42.7 ± 3.9 | -42.6 ± 1.9 | 0.388 ± 0.024 | 67.4 ± 2.3 |

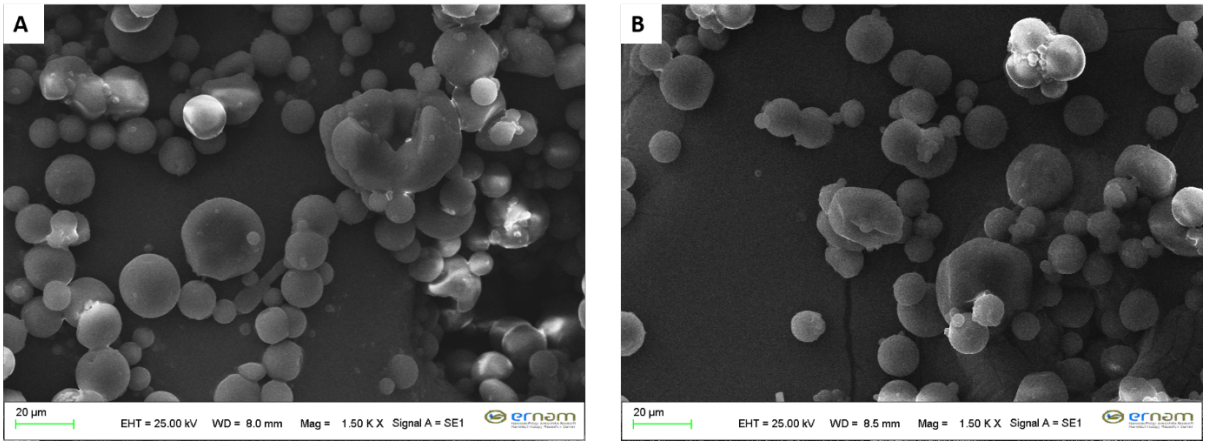


**Figure 2.** SEM photo of blank PLGA MCs (A) and (B) FB-loaded PLGA MCs.

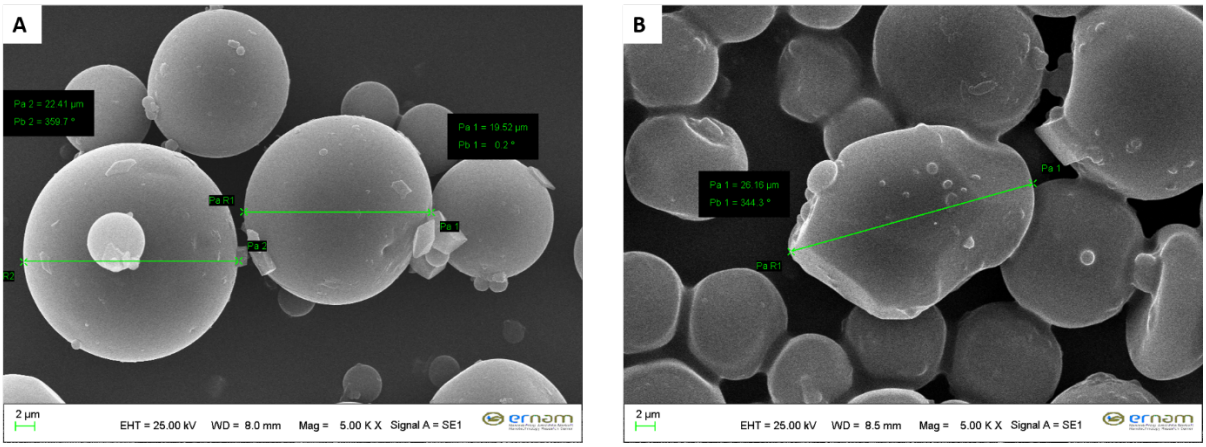


**Figure 3.** SEM image that was magnified considerably to allow for a closer look at the microsphere surface. Blank PLGA MCs(A) and (B) FB-loaded PLGA MCs

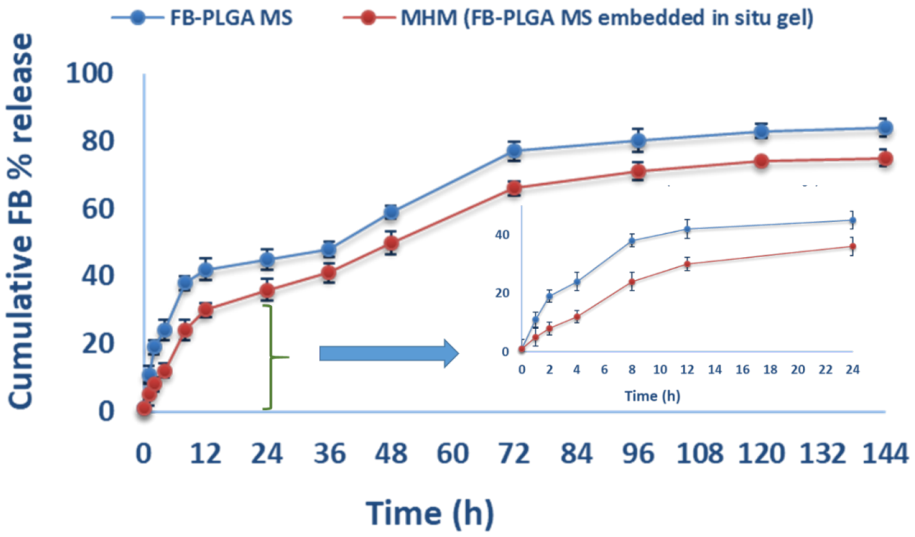


**Figure 4.** Cumulative FB release from microspheres and in situ gel formulation with FB-loaded microspheres (n=3)

**Table 3.** Results of *in vitro* characterisation of *in situ* gels.

| Formulation | pH (±SD) | Gelation temperature (°C±SD) | Gelation time (s) | Viscosity (cP) = 25 °C | Viscosity (cP) = 35 °C | Clarity |
|---|---|---|---|---|---|---|
| A1 | 7.02±0.01 | 41±0.5 | 51±0.5 | 272±25 | 842±98 | + |
| A2 | 7.00±0.02 | 40±0.3 | 44±0.9 | 333±44 | 978±123 | + |
| A3 | 6.97±0.03 | 39±0.4 | 38±0.3 | 359±41 | 1045±152 | + |
| A4 | 6.96±0.02 | 38±0.6 | 43±0.5 | 352±39 | 7872±141 | + |
| A5 | 6.93±0.01 | 36±0.7 | 38±0.7 | 384±56 | 10088±212 | + |
| A6 | 6.9±0.02 | 35±0.1 | 33±0.2 | 422±38 | 11042±247 | + |
| A7 | 6.89±0.06 | 30±0.4 | 35±0.1 | 425±55 | 16642±432 | + |
| A8 | 6.82±0.03 | 29±0.4 | 32±0.3 | 497±68 | 19482±479 | + |
| A9 | 6.78±0.04 | 26±0.2 | 27±0.2 | 944±87 | 22813±574 | + |

## DISCUSSION

### *In vitro* characterisation of FB-loaded PLGA MCs

The encapsulation of FB in microspheres may cause an increase in particle sizes of the drug-containing microspheres. There are trials employing microspheres made for treating eye infections ranging in size from 8.5 µm to 25 µm. (Ding et al., 2018). Hence, the particle size of the obtained FB-loaded PLGA microspheres was suitable for an ocular controlled release system.

The drug release from the created microspheres experienced a burst impact, as observed by approximately 40% release at the conclusion of the eighth hour. From the eighth to the third hour of this time, a slower release was noticed, which was attributed to ongoing diffusion-based release from the polymeric microspheres. A second rapid release profile with a 36-to 72-hour duration was found. This was understood to mean that the accelerated breakdown of the polymeric matrix boosted drug release. The expansion of microsphere pores and activation of new diffusion routes are also relevant in this context (Siep-

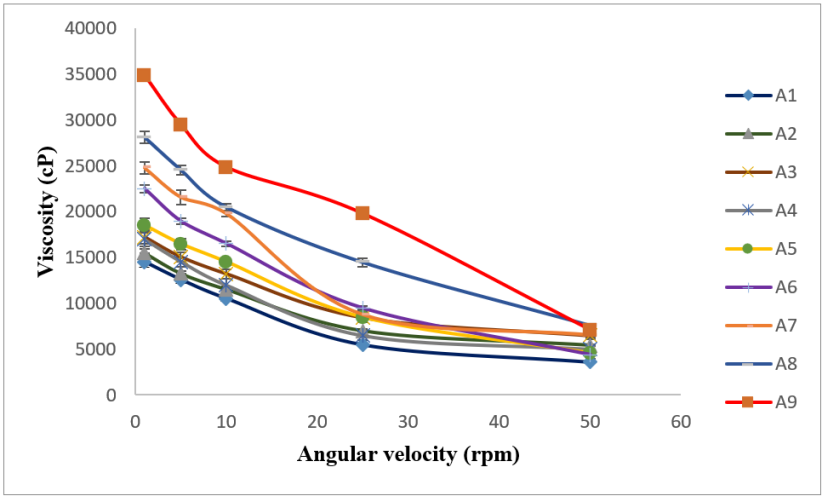


**Figure 5.** Rheological characteristics of in situ gelling systems' - (n=3)

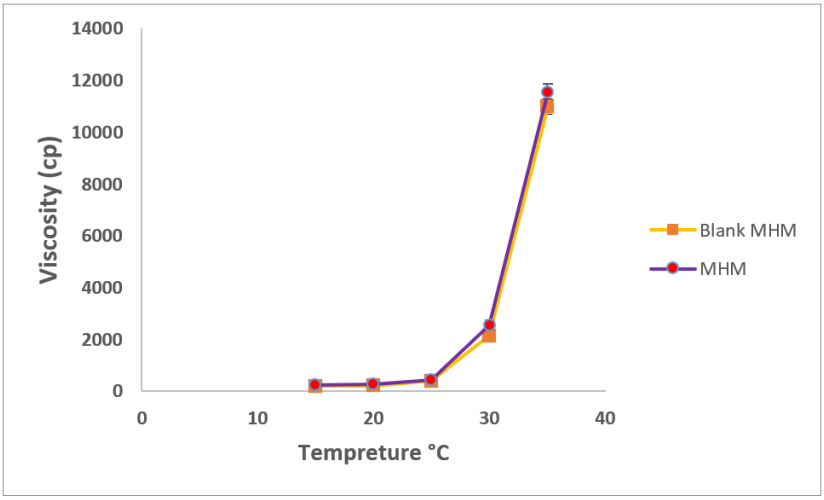


**Figure 6.** Viscosity profiles of MHM (n=3) at different temperatures (n=3).

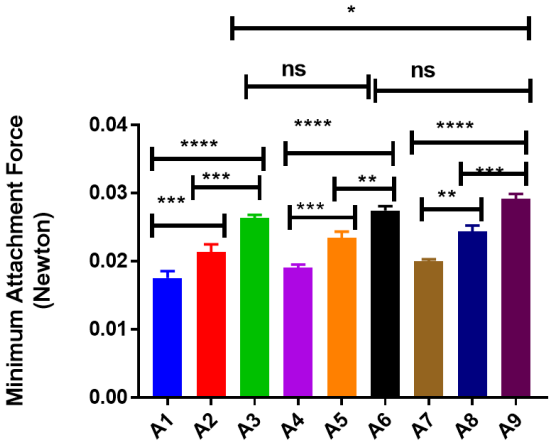


**Figure 7.** Mucoadhesion study results for ın situ gels (n=3).

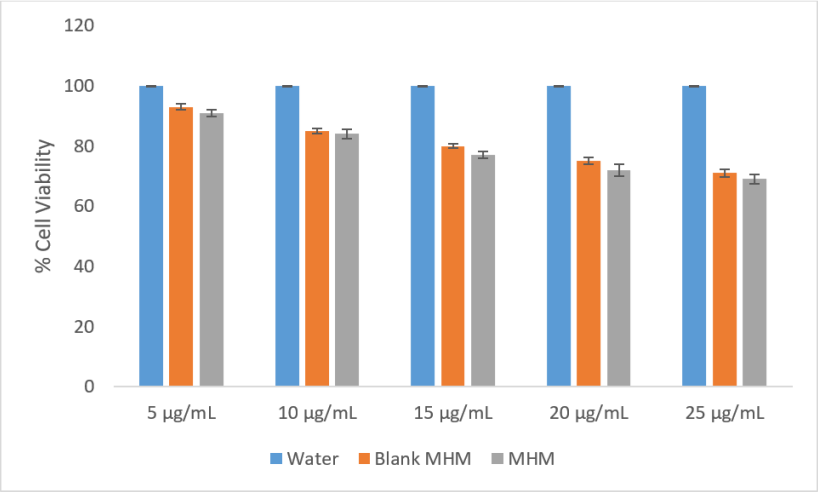


**Figure 8.** The biocompatibility of water, blank MHM, and MHM was investigated using an MTT assay with ARPE-19 cells (n=3)

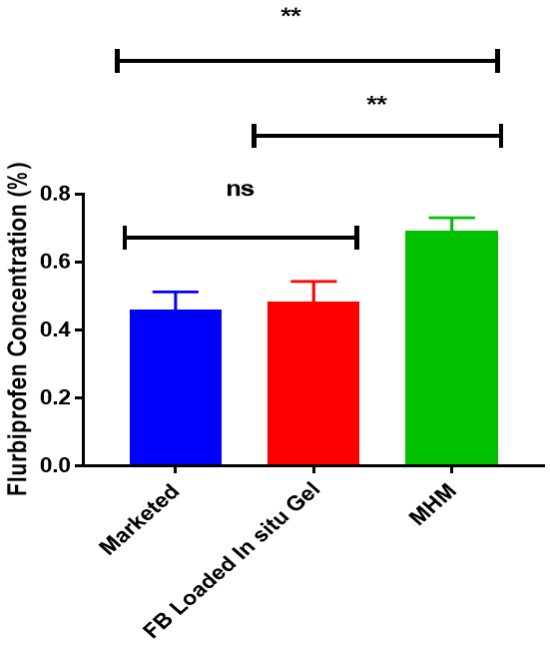


**Figure 9.** Cumulative corneal transport of commercially available product and formulations prepared in this study (n=3).

mann, Faisant, & Benoit, 2002). From the 72$^{nd}$ to the 144$^{th}$ hour, a more regulated release profile was observed as the drug concentration in the microspheres decreased with time, and the bursting effect of the degradation process subsided. The profile began to plateau at the 96$^{th}$ hour. After examining the release profile, it was discovered that at the end of the fifth day, approximately 80% of the encapsulated drug had been released from the polymeric matrix. A regulated release was observed because of the drug's diffusion, breakdown, and polymer degradation over the course of six days (Yasukawa, Kimura, Tabata, & Ogura, 2001).

### *In vitro* Gel Characterisation

These findings show poloxamer gel's thermosensitivity is strongly influenced by the concentration; a higher PX 407 concentration accelerated gelation at a lower gelation temperature. To decrease the polymer concentration of the in situ gel while enhancing the gelling properties and rheological behaviour, the interaction of in situ gelling polymers with HEC as a viscosity-modifying agent was explored. A9 (PX 407 and HEC) exhibited optimal gelation at concentrations of 17% and 1.5%, respectively. It is hypothesised that HEC molecule entanglement and

substantial hydrogen bonding result in firmly aligned gel formations within Pluronic micelle routes.

Precise formulations must be used for ocular applications. Despite the efficacy of drug delivery methods, such as ointments, patient compliance is low because they cause blurred vision. Regardless of the effectiveness of the medication, outcomes cannot be reached if the patient does not follow instructions. For this reason, the compositions intended for ocular use must be transparent (Patel, Cholkar, Agrahari, & Mitra, 2013).

According to a literature review, a pH range of 4–8 is appropriate for eyes (Patel et al., 2013). Ophthalmic thermo reversible gels' sol-gel transition temperatures of ophthalmic thermo-reversible gels are between 25 and 34°C, making them appropriate for ocular delivery. When t thermosensitive formulation's gelation temperature is less than 25°C, a gel may form at room temperature. When > 34°C, a liquid dosage form persists at the ocular surface temperature, causing the formulation to drain from the eyes. When the two poloxamer grades are combined, it is possible to control the gelation temperature to remain within the permissible range (25-34°C) (Abd Elhady, Mortada 6Awad, & Zaki,- 2003).

Two polymer grades were researched and employed to form in situ forming gels to select formulations with an adequate sol-gel transition temperature and the lowest total poloxamer concentration. The formulations of P407/P188 (15/8 %, wt/wt) for the in situ-forming gel of FB-loaded MSs produced satisfactory results. The in situ gel formation temperatures for all FB-loaded MS formulations were between 26°C and 41°C, particularly for A5 and A6, which are suitable for ocular administration. The data in Table 3 show this.

To generate poloxamer, an amphiphilic synthetic copolymer, two hydrophilic blocks of polyethylene oxide (PEO) are sandwiched between two hydrophobic blocks of polypropylene oxide (PPO). Amphiphilic block copolymer molecules form small micellar subunits when mixed together in aqueous liquids. Polymer molecules join to form a vast micellar cross-linked network when concentrations above a particular level, known as the critical micelle concentration, are reached (Heybet Kerem Polat et al., 2023). The temperature also significantly affects micelle formation. The PEO and PPO blocks are hydrated when the temperature falls below the critical micelle temperature. In aqueous solutions, PPO is also largely soluble. The PPO chains interact hydrophobically as the temperature rises, becoming dehydrated and less soluble than the PEO chains. It is difficult for these micelles to disperse in the solution independently, so they interact and tangle with other micelles. Because of this entanglement, a three-dimensional network structure was created (Wanka, Hoffmann, & Ulbricht, 1994), and elation required higher temperature at lower P-407-127 concentrations.

The gelation temperature of the in situ-forming gels was lowered by adding mucoadhesive polymers like HEC, as shown in Table 3. The transition temperature gradually decreased when the polymer concentration was increased from 0 to 1 and 1.5%. Such mucoadhesive polymers' ability to bind to polyoxyethylene chains found in poloxamer molecules may explain their ability to reduce gelation temperatures. Including mucoadhesive polymers, which allow attachment of the formulations to the corneal mucin, would significantly minimise the drainage of ophthalmic formulations from the pre-corneal surface. As a result, dehydration is encouraged, enhancing intermolecular hydrogen bonding and the entanglement of nearby molecules, resulting in gelation at lower temperatures (Gilbert, Richardson, Davies, & Hadgraft, 1987).

The system is expected to gel instantly or quickly upon exposure to its gelation temperature, preventing rapid evacuation by tear fluid (Venkatesh, Kamlesh, & Kumar, 2013). The gelation times of A1 and A9 were approximately 51 and 27 s, respectively (Table 3). Additionally, A9, which exhibited higher concentrations of PX 407 and HEC, required less gelation time. This finding shows that shorter gelation times are associated with reduced effective sol-gel transition temperatures caused by increased pluronic and HEC concentrations (Ricci, Lunardi, Nanclares, & Marchetti, 2005).

**Determination of the Mucoadhesive Force**

The formulations comprising mucoadhesive polymers, such as HEC, considerably improved the mucoadhesive force of the ciprofloxacin HCl in situ-producing gels. The mucoadhesive force dramatically increased with increasing polymer concentrations ($p < 0.0001$).

Mansour et al. (Mansour, Mansour, Mortada, & Abd ElHady, 2008) developed ciprofloxacin-loaded poloxamer in situ gels. Different concentrations of HPMC and HEC were used to increase the in situ mucoadhesion of the gels. Because of their mucoadhesion study, they determined that the use of increasing concentrations of HPMC and HEC significantly increased mucoadhesion. In another study, fluconazole-loaded in situ gels were prepared and HEC was used to increase the viscosity of the gels. In a mucoadhesion study, it was determined that the mucoadhesive properties of HEC increased with increasing doses (Gonjari et al., 2009).

**Rheology and viscosity**

According to a literature review, the recommended viscosity for effective ocular delivery is 50–50,000 cp. As the formulation spends more time in the dead end region of the eye, its bioavailability will increase in proportion to the increase in viscosity. To determine the viscosity values of all formulations, 10 rpm measurements at 25°C and 35°C. The data analysis showed that the viscosity changed depending on the polymer content (Table 3). The significance of the effect of polymer concentration on viscosity is illustrated by the above example. The above example shows the significance of the effect of polymer

concentration viscosity. The results were consistent, according to a literature review (Fathalla et al., 2017).

When using excessively viscous in situ gels, several problems could arise; however, when using in situ gels that are low in viscosity, tears can be cleared from the surface of the eye quickly. High-viscosity ocular formulations should be avoided because they frequently leave a noticeable residue on the side of the eyelid where they are administered. Adopting in situ gel formulations with pseudo-plastic behaviour makes it feasible to decrease the adverse effects of ocular reflexes, such as blinking, in the formulation. The pseudo-plastic flow of the formulation allows for a more pleasant application and longer corneal contact time. The viscosity increased as the PX 407 content increased from 12% to 18% (wt/wt). This may be explained by the fact that the poloxamer is a nonionic triblock copolymer composed of polyoxyethylene, polyoxypropylene, and polyoxyethylene that aggregates into micelles at 34°C because of the dehydration of the polymer blocks with temperature. Micellar packing and enlargement lead to gel development, and the gel becomes more twisted at greater poloxamer concentrations. These micelle entanglements prevent the micelles from simply separating from one another, which explains the hard and viscous gel with high poloxamer concentrations is hard and viscous (Cabana, Aıt-Kadi, & Juhász, 1997). We can infer that higher concentrations of the mucoadhesive improve the viscosity of the formulations by examining the impact of various mucoadhesive concentrations, such as HEC, on the viscosity of the poloxamer in situ-forming gels. The theory that block copolymer PX407 thermosensitive gels are produced via hydrogen bonding in aqueous systems, which is brought about by the attraction between the oxygen atom of the poloxamer aether and the protons of water, may explain this. The addition of substances containing hydroxyl groups, such as the studied cellulose derivatives, is anticipated to increase the number of hydrogen bonds, thereby increasing the measured viscosity of the resulting formulations.

When the in vitro characterisation results were examined, it was decided that the A6 formulation could be applied ocularly due to its gelling temperature, viscosity, and mucoadhesive properties. After this stage, the A6 formulation code was changed to MHM, and the study continued with this formulation.

Rheological experiments were conducted at various temperatures to determine the thermoreversible characteristics of the formulations under accelerated thermal fluctuations. The formulations were subjected to a heat cycle between 10°C and 40°C before rheological testing. The results showed that rising temperature caused the formulation viscosity to increase. At 25°C, the viscosity of MHM was found to be 436±35 cP, whereas it increased to 11542±321 cP at 35°C.

### *In vitro* Release

The overall release percentage of FB-loaded MSs was 74 ±0.8%. Fig. 8 indicates that from 0 h to 8 h, the cumulative release percentage of FB-loaded MSs was 39±0.74%, whereas that of MS-loaded in situ gel was 23± 0.48. It can be seen that there is a difference in burst effect between in situ gels and microspheres. Compared with MHM, FB-loaded MSs had a significantly higher burst release percentage.

It is thought that this difference in the burst effect is caused by the in situ gel. The following two actions may have been included in the in situ gel release profiles: First, the nanoparticles were simultaneously freed from the skeletal carrier as the in situ gel slowly decomposed. The second stage was the gradual release of the medication from the PLGA nanoparticles. It was now clear why there were differences between the release characteristics of the two formulations. The gel initially prevented MSs loaded with FB from contacting the release medium. An increase in the number of microparticles discharged as the gel gradually degraded. All nanoparticles were released at a rate comparable to that of FB-loaded MSs 24 h after the gel had dissolved, indicating their release behaviour was dependent on a diffusion process. After examining the literature, it is possible to show that the results are consistent (Morsi, Ghorab, Refai, & Teba, 2016).

Hu et al. determined that when they developed a rifampicin-loaded PLGA microsphere and loaded it onto an in situ gel containing sodium alginate, the burst effects were lower due to the dual structure of the in situ gels (Hu, Feng, & Zhu, 2012). Beg et al. loaded moxifloxacin-loaded nanoparticles, which they produced in another study, onto in situ poloxamer gels. Because of the release study, we determined that the burst effect of nanoparticle-loaded in situ gels was lower than that of drug-loaded nanoparticles. In another study, PLGA nanoparticles loaded with fluorometholone were prepared for use in ocular inflammation and loaded in situ gels. The release study determined that the burst release of in situ gels was significantly lower than that of nanoparticles (Gonzalez-Pizarro et al., 2019).

### Release kinetics

The results obtained from *in vitro* release studies were fitted into various mathematical models, including first-order and zero-order Higuchi, Korsmeyer, Hollenberg, Baker-Lonsdale, Peppas-Sahlin, and Weibull models, to investigate the FB release kinetics. DDSolver software was used for the best-fitted mathematical model with the highest $R^2$, $R^2_{adjusted}$, MSC, and AIC values. The release kinetic parameters are listed in Table 4.

**Table 4.** Release kinetic modelling and results of FB-loaded PLGA- and FB-loaded in situ gels

| Model and equation / Formulation | | Evaluation criteria | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Parameter | | $R^2$ | $R^2_{adjusted}$ | AIC | MSC | n/m* |
| **Zero-order** | FB-PLGA MS | k0 | 0.776 | 0.3932 | 0.3932 | 114.7558 | 0.3457 | - |
| F=k0*t | MHM in situ gel | k0 | 0.679 | 0.6842 | 0.6842 | 105.3656 | 0.9987 | - |
| **First-order** | FB-PLGA MS | k1 | 0.022 | 0.8160 | 0.8160 | 99.2406 | 1.5392 | - |
| F=100*[1-Exp(-k1*t)] | MHM in situ gel | k1 | 0.014 | 0.9179 | 0.9179 | 87.8525 | 2.3459 | - |
| **Higuchi** | FB-PLGA MS | kH | 8.131 | 0.9106 | 0.9106 | 89.8625 | 2.2606 | - |
| F=kH*t^0.5 | MHM in situ gel | kH | 6.962 | 0.9771 | 0.9771 | 71.2804 | 3.6206 | - |
| **Korsmeyer-Peppas** | FB-PLGA MS | kKP | 15.742 | 0.9774 | 0.9753 | 73.9900 | 3.4815 | 0.347 |
| F=kKP*t^n | MHM in situ gel | kKP | 8.650 | 0.9820 | 0.9803 | 70.1525 | 3.7074 | 0.450 |
| **Hopfenberg** | FB-PLGA MS | kHB | 0.000 | 0.8160 | 0.7993 | 101.2424 | 1.3852 | - |
| F=100*[1-(1-kHB*t)^n] | MHM in situ gel | kHB | 0.000 | 0.9178 | 0.9104 | 89.8605 | 2.1914 | - |
| **Baker-Lonsdale** | FB-PLGA MS | kBL | 0.002 | 0.9674 | 0.9674 | 76.7531 | 3.2690 | - |
| 3/2*[1-(1-F/100)^(2/3)]-F/100=kBL*t | MHM in situ gel | kBL | 0.001 | 0.9857 | 0.9857 | 65.1459 | 4.0925 | - |
| **Peppas-Sahlin** | FB-PLGA MS | k1 | 14.752 | 0.9787 | 0.9744 | 75.2343 | 3.3858 | 0.413 |
| F=k1*t^m+k2*t^(2*m) | MHM in situ gel | k1 | 6.036 | 0.9901 | 0.9882 | 64.3082 | 4.1570 | 0.621 |
| **Weibull** | FB-PLGA MS | β | 0.530 | 0.9744 | 0.9693 | 77.5887 | 3.2047 | - |
| F=100*{1-Exp[-((t-Ti)^β)/α]} | MHM in situ gel | β | 0.631 | 0.9895 | 0.9874 | 65.1102 | 4.0953 | - |

The data obtained from *the in vitro* drug release of FB-PLGA were fitted to the Korsmeyer model with the highest R2, R2adjusted, MSC, and AIC values. Korsmeyer-Peppas model, also known as "Power low," portrays the release mechanisms, including the diffusion of water into the matrix and swelling or dissolution of the matrix, using the "n" value in the Korsmeyer-Peppas. The "release exponent n" value elucidates the drug-release mechanism. If the "n" value is less than 0.45, drug release corresponds to Fickian diffusion. When these data were examined using the Korsmeyer-Peppas model, FB release from PLGA NPs was compatible with Fickian diffusion. On the other hand, the Peppas-Sahlin model exhibited superior describing the release mechanisms of FB from MHM in situ gels. "m" diffusional exponent is equal to the "n" value in the Korsmeyer model. If "m" is less than 0.45, then Fickian diffusion. If "m" is between 0.45 and 0.85, drug release depends on non-Fickian diffusion. If m = 0.85, drug release occurs through case II transport (Supramaniam, Adnan, Mohd Kaus, & Bushra, 2018). The "m" value was examined; it was 0.621 for the MHM-in situ gel. It was observed that there was non-Fickian diffusion considering the diffusional exponent parameter of the Peppas-Sahlin model.

**Biocompatibility studies (MTT assay)**

The cytotoxicity of ARPE-19 cells, as measured using in situ gel microscopy, was found to be acceptable. Consequently, the created in situ gel system is biocompatible. Applications were made to the in situ gel cell line at different concentrations. It was determined that cell viability decreases with increasing concentrations. Although there was no significant change in the positive control group at the lowest dose, a statistically significant change was observed at the highest dose ($P < 0.01$). Another eye study demonstrated that the cytotoxicity of flurbiprofen changed in a dose-dependent manner (Vasconcelos et al., 2015).

One of the numerous polymeric drug delivery vehicles is PLGA, which is crucial in applications that integrate targeting, imaging, diagnostics, and therapy. Furthermore, this polymer readily hydrolyses to monomers like lactic acid or glycolic acid, which are then excreted from the body via normal metabolic pathways. There was no discernible difference between the formulations of the blank in situ gel (loaded just with PLGA microspheres) and FB microsphere-filled in situ gel after evaluation. When the literature is examined, it is seen that there are similar results (Öztürk, Yenilmez, Şenel, Kıyan, & Güven, 2020).

### *Ex vivo* Transport Studies

In situ gel formulations were used in transport investigations of sheep corneal tissue for a 2-hour period together with a commercially available medication (suspension), and the tissue was not harmed (Aytekin et al., 2020). Similar findings were often reported in the literature review. Veiga et al. In a study they conducted, they produced Nepafenac-loaded microparticles. In their ex vivo study, they determined that the microparticles loaded with Nepafenac passed more than the market preparation (Lorenzo-Veiga, Diaz-Rodriguez, Alvarez-Lorenzo, Loftsson, & Sigurdsson, 2020).

## CONCLUSION

In this study, flurbiprofen-loaded PLGA microspheres were formed and evaluated. Additionally, the study investigated adding microspheres into a thermosensitive in situ gel preparation for ophthalmic delivery. A significantly modified single-emulsification/solvent evaporation process (O/W), proper particle size, zeta potential, and sustained release were used to prepare FB-loaded PLGA microspheres. The elucidation of the in vitro release kinetics of FB-PLGA MSs as mathematical modelling has also been demonstrated by our study. Our study has also demonstrated the elucidation of the *in vitro* release kinetics of FB-PLGA MSs as mathematical modelling. Furthermore, a release kinetic analysis was performed for the gel system containing MS. After the comprehensive characterisation study, we determined that FB-PLGA MS and gel systems fit different mathematical models. A6 (MHM) was therefore selected to be incorporated into a thermosensitive in situ gel prepared with 15% PX-407 + 8% PX-407 + 1.5% HEC, which displayed the optimum gelling capacity, a mucoadhesive structure, and sustained release and good rheological behaviour. - MTT analysis of the ARPE-19 cell line showed that the in situ gel formulation was biocompatible. In addition, the ex vivo corneal transition study showed that MHM had higher corneal penetration than that of the marketed product. The optimized his formulation could be considered a viable ocular drug delivery method for treating postoperative inflammation due to its decreased administration interval.

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