

A.Dian Permana-6_Usulan.pdf

by

Submission date: 09-Dec-2022 07:59AM (UTC+0700)

Submission ID: 1975830034

File name: A.Dian Permana-6_Usulan.pdf (3.98M)

Word count: 7389

Character count: 39043



Enhanced localization of cefazoline sodium in the ocular tissue using thermosensitive-mucoadhesive hydrogels: Formulation development, hemocompatibility and *in vivo* irritation studies

Muhammad Al Fiqri^a, Alhidayah^a, Nirmayanti^a, Ummu Athiyah^a, Patricia Layadi^a, Tamara Gabriela Angeleve Fadjar^b, Andi Dian Permana^{a,*}

^a Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
^b Faculty of Medicine, Hasanuddin University, Makassar, 90245, Indonesia

ARTICLE INFO

Keywords:
Cefazoline
Bacterial keratitis
Mucoadhesive-thermosensitive *in situ* gel

ABSTRACT

Bacterial keratitis is an eye infectious disease which became a global concern due to its impact to visual impairment in worldwide. Cefazolin (CFZ) is the first-line drug for the treatment of bacterial keratitis and only available in the drop dosage form. However, the administration of eye drops results in lack of bioavailability, which is below 5%. Therefore, an innovation is required in an attempt to overcome these problems. In this study, the development of a mucoadhesive-thermosensitive gel *in situ* preparation was carried out. A combination of Pluronic F127 and F68 was used as a thermosensitive agent. To increase the contact time, a mucoadhesive agent, namely hyaluronic acid was added to the formulation. Several steps of evaluation were performed to ensure that the developed formula possessed desired characteristics, including determination of gelation temperature, pH test, viscosity test, rheology test, mucoadhesive test, drug content test, *in vitro* drug release test, *in vivo* irritation test on experimental animals, *ex vivo* permeation test, and hemolysis test. The results that the formulations developed exhibited desired characteristics, with gelation temperatures of around 37 °C. The formulation could also control the release and improve the localization of CFZ in the ocular tissue compared to control solution. Furthermore, the incorporation of CFZ into this approach did not change the antimicrobial activity of CZ against *Pseudomonas aeruginosa*. Importantly, no toxicity and irritation were observed after the application of this approach. However, further research is needed to evaluate the pharmacokinetic and pharmacodynamic in the appropriate animal models.

1. Introduction

Bacterial keratitis is an inflammatory process caused by a bacterial infection. Approximately 64.6% of keratitis cases are caused by bacterial infection. Use of contact lenses has been reported to be the main risk factor of this disease [1]. Previous study has reported that incidence of microbial keratitis with stromal infiltration and ulcers due to the use of contact lenses was 38,7% [2]. Contact lenses block the cornea from oxygen, tears, and ocular secretions; which can affect the occurrence of infection or inflammation of the corneal layer, resulting in an impaired vision. The symptoms and signs of bacterial keratitis are pain, redness, blurred vision, discharge, corneal infiltrates, ulceration, photophobia, and inflammation of anterior chamber of the eye. The treatment of this disease must be supported by a medical history, physical

examination and slit-lamp examination [3].

Topical antibiotics remain the first-line treatment for bacterial keratitis [4]. Cefazoline (CFZ) is a first-generation cephalosporin antibacterial used for the treatment of infections, inhibiting bacterial cell wall synthesis. CFZ is given in the form of eye drops at a dose of 50 mg/mL [3]. In ocular drug application, the presence of precorneal factors and anatomical barriers have limited the bioavailability of the eye preparations. Precorneal factors include solution drainage loss from blinking, tear film formation, and increased tear secretion. Considering all these precorneal factors, the contact time of the ocularly applied preparations is very low and, therefore, only <5% can penetrate the intraocular tissues [5]. Eye drops are the type of preparation that are most often applied topically to the ocular surface to treat external ocular infections. However, due to the protective mechanism of the eye, it

* Corresponding author. Faculty of Pharmacy, Hasanuddin University, Indonesia.
E-mail address: andi.dian.permana@farmasi.unhas.ac.id (A.D. Permana).

<https://doi.org/10.1016/j.jddst.2022.103763>

Received 9 July 2022; Received in revised form 7 August 2022; Accepted 29 August 2022

Available online 5 September 2022

1773-2247/© 2022 Elsevier B.V. All rights reserved.

results in very poor bioavailability of the drug [6]. Furthermore, it also causes low drug permeation through the ocular tissue and because the eye pocket has a limited capacity, the amount of drug absorbed by the ocular tissue is unknown [7].

The *in situ* gel delivery system is concerned with the conversion of the liquid state of the formulation (solution) into gel at the site of application under certain physiological conditions. Many factors regulate gel formation *in situ* including temperature, pH, solvent exchange, ionic cross-linking and ultraviolet radiation [8]. Thermosensitive *in situ* gel is a viscous liquid that can thermally turn into a gel after entering the ocular physiological fluid [9]. The heat-sensitive *in situ* gelling system is in a liquid state at room temperature (25 °C) and undergoes a transition to a gel at a temperature value close to physiological body (32 °C–37 °C), depending on the site of administration [10]. The main advantages of *in situ* gel are delayed and controlled drug delivery, and reduced or absent blurred vision in comparison with eye ointments. Another advantage of the *in situ* gel system over eye drops and ointments is the increased bioavailability of the drug due to increased pre-corneal contact. In addition, the *in situ* gel system may be more convenient than insoluble or soluble insertions [11].

The polymer commonly used in thermosensitive *in situ* gel systems is Poloxamer (Pluronic®) [12]. Pluronic F127 is the type of Poloxamer that has been most often used in ophthalmic delivery systems [13]. It was found that *in situ* gel formulation using single Poloxamer F127 had 27.2 ± 0.4 °C of gelation temperature. This temperature was lower than the physiological temperature of the eye. The gelation temperature of Pluronic® F127 can be increased by combining Pluronic F127 with other types of Pluronic [14]. To the best of our knowledge, until now, there has been no research that examines Pluronic® F68 as a single polymer or in combination with other Pluronic for *in situ* gel preparations for the eye containing CFZ.

To enhance the contact time of the *in situ* gel with the ocular tissue for potential increased bioavailability, a mucoadhesive agent is needed in the preparation [15]. One of the mucoadhesive agents that most recommended is hyaluronic acid (HA). HA has the advantage when formulated into ophthalmic preparations, mainly due to its ability to increase contact time. Importantly, HA has excellent biocompatibility and biodegradability in the eye. This polymer can even protect the corneal epithelium from dehydration and reduce the inflammatory response to dehydration. HA can lubricate the ocular surface to prevent dehydration [16]. In this study, a thermosensitive mucoadhesive *in situ* gel formulation from CFZ was carried out using a combination of Pluronic® F127 and F68 with HA as mucoadhesive agent.

2. Material and methods

2.1. Materials

Cefazolin sodium salt (CFZ) (purity 89.1–110.1%) was purchased from Sigma Aldrich Pte Ltd. (Singapore, Singapore). Pluronic® F127 and F68 used in this study were generous gifts by BASF Indonesia (Jakarta, Indonesia). Other chemicals used in this study were analytical grade.

2.2. Design of formulation

The formulation of the thermosensitive *in situ* gel was produced by dissolving Pluronic® F127 and Pluronic® F68 at a temperature of 4 °C until fully dissolved [17]. The compositions of the formulation are depicted in Table 1. The mucoadhesive agent was prepared by carefully weighing the HA, then dissolved in distilled water and carefully heated on an electric stove until dissolved. After the hyaluronic was completely dissolved and placed at room temperature, it was mixed with the thermosensitive gel formulation. CFZ and benzalkonium chloride were weighed carefully and added to the basic mixture of the mucoadhesive thermosensitive *in situ* gel formulation, then homogenized. Furthermore, several evaluations of the mucoadhesive thermosensitive *in situ*

Table 1
Design of CFZ mucoadhesive-thermosensitive formulation.

Ingredients %(w/w)	F1	F2	F3	F4	F5
CFZ Sodium	0,35	0,35	0,35	0,35	0,35
Pluronic® F127	15	15	15	15	15
Pluronic® F68	5	5	5	5	5
HA	0,25	0,2	0,15	0,1	0
Benzalkonium Chloride	0,01	0,01	0,01	0,01	0,01
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

gel formula were carried out.

2.3. Evaluation

2.3.1. Mucoadhesive-thermosensitive *in situ* gelation temperature study

Initially, 2 mL of each formula was put into test tube. The test tube was put into water bath at 20 °C and then the temperature of the bath was increased slowly until 65 °C. The test tubes containing each formula were observed visually after a 1 °C increase in temperature. The gelation temperature was recorded as the temperature at which the gel did not move when the test tube was inverted to 90° [18].

2.3.2. Mucoadhesive study of CFZ *in situ* gel formulation

Determination of the mucoadhesive test was carried out using a modified balance tool, where the left arm of the balance was attached to two layers of pig eye tissue that were applied with mucoadhesive thermosensitive *in situ* gel formula. On the other hand, on the right arm of the balance tool, 1 g of the metal weight was added every 30 s until ocular tissue was separated from other tissue [19,20].

2.3.3. pH evaluation

The pH value was assessed using a digital pH meter.

2.3.4. Viscosity rheology study of gel *in situ* formulation

Rheological was determined by using viscometer (Brookfield, USA). In an attempt to assess the flow properties, we calculated the velocity against the viscosity. The viscosity was measured on three different temperatures, namely 25 °C, 37 °C, and 4 °C.

2.4. Drug content determination of CFZ in mucoadhesive-thermosensitive *in situ* gel formulation

Each formula was carefully weighed and dissolved in simulated tear fluid (STF) to achieve a final concentration of 1 mg/mL. All formulations were then centrifuged for 15 min and the supernatant was measured using UV-vis spectrophotometry at a wavelength of 233.4 nm [21].

2.5. *In vitro* drug release study

In this study, the release study of CFZ from mucoadhesive-thermosensitive *in situ* gel formulation, a dialysis method was utilized. The experiment was carried out at 37 °C at 100 rpm. The dialysis membrane used was soaked with STF prior to the experiments. Afterwards, the amount of each formula was calculated to be equivalent to 10 mg of CFZ, then put into a dialysis membrane and both sides of the membrane were closed with airtight clamps. The membrane was put into 100 mL of STF inside the glass bottle. The media (1 mL) was then sampled at a time range of 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, and 24 h and replaced with 1 mL of fresh STF. The release kinetic profile was determined using the following equations [22].

Zero Order Kinetics: $C_t = C_0 + k_0t$

First Order Kinetics: $\ln C_t = \ln C_0 + k_1t$

M. Fiqri et al.
Higuchi Model: $C_t = k_H t$

Korsmeyer–Peppas Model: $C_t = kKPt^n$

Hixson–Crowell Model: $C_t^{3/2} = C_0^{3/2} k_{HC} t$

where C_t is the amount of CFZ released at time t , C_0 is the initial concentration of CFZ in STF (at $t = 0$), k_0 is constant of zero-order kinetics, k_1 is constant of first-order kinetics, k_H is constant of Higuchi model, k_{KP} is constant of Korsmeyer–Peppas model, and k_{HC} is constant of Hixson–Crowell model.

2.6. Ex vivo permeation study of CFZ mucoadhesive-thermosensitive in situ gel formulation

Pig cornea was taken carefully using forceps and cut with about 5–6 mm of scleral tissue around it, then washed with 0.9% NaCl solution. Then the cornea was placed between the donor compartment and the Franz diffusion cell receptor. The receptor compartment with a capacity of 10 ml was filled with STF and stirred at 100 rpm stirring on a magnetic stirrer. The temperature was maintained at 37 ± 0.5 °C. The formulation was applied into the donor compartment. At 0.25, 0.5, 1, 2, 3, 4, 5, 6-, 7-, 8-, and 24-h, samples (1 mL each) were sampled and replaced with equal volumes of receptor medium. The absorbance was measured at a wavelength of 233.4 nm. Then, the concentration in the sample was calculated [18]. Afterwards, the fluxes (J , $\mu\text{g}/\text{cm}^2/\text{h}$) and the permeability coefficients (K_p , cm/h) were determined as previously described [23,24].

2.7. Hemolysis study

Fresh blood was collected, anticoagulated, and then stored at room temperature [25]. The red blood cells were collected and diluted with PBS pH 7.4 to achieve a final concentration of 10% v/v. Then, the test formulation was incubated with the red blood cells. The hemolysis percentages of the hemoglobin from the blood were measured as reported previously [20,26].

2.8. In vitro antibacterial activity

2.8.1. Culture of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (ATCC® 9027) was purchased from LGC Standards, Middlesex, UK. The bacterial were cultured in tryptic soy broth (TSB) media at 37 °C overnight. The cultured bacterial were diluted in fresh TSB to obtain a final bacterial number of 1×10^8 CFU/mL. The optical density was measured at 550 nm.

2.8.2. In vitro antibacterial activity of CFZ in mucoadhesive-thermosensitive in situ gel formulation

Agar diffusion method was used to assess the antibacterial activity of CFZ following the incorporation into mucoadhesive-thermosensitive in situ gel formulation. The bacterial suspension of *Pseudomonas aeruginosa* was cultured in tryptic soy agar (TSA) using spreading method. Following this, paper discs containing CFZ solution, CFZ thermosensitive formulation, water and free disc were placed on the top of the bacterial culture. The concentration of CFZ used was equal to 5 $\mu\text{g}/\text{mL}$. The media was incubated at 37 °C overnight and the inhibitory zone was calculated using digital vernier calipers.

2.9. In vivo irritation study on rabbit's eye

This study was carried out under the permission from Health Ethical Committee of Hasanuddin University, Indonesia. In this study, the application of the formulation was performed in the rabbit's eyes without the use of anesthesia. Several assessments of signs of irritation including redness, swelling, cloudiness, edema, bleeding, and discharge were observed at regular intervals during the 3 days of treatment. Draize

eye irritation test was used to observe changes in the cornea, conjunctiva, and iris in the rabbit's eyeball after exposure to test substances. In this study, 0.1 mL of the formulation was applied on the cornea and conjunctival sac of one rabbit's conscious eyeballs. The irritation of the eye was assessed using Maximum Average Score (MAS) [27].

2.10. Histopathological evaluation

In the end of the experiments, the rabbits were dissected for their ocular tissue collection and preserved in 10% neutral buffered formalin. Then, the tissue was placed in paraffin wax. Sections with a thickness of 4 μm were cut and stained with Hematoxylin and Eosin (H&E) and then observed using a microscope, as previously described [28].

2.11. Statistical analysis

All data were reported as mean \pm standard deviation (SD) and statistically analyzed using one-way ANOVA and Tukey's test with GraphPad Prism version 6.0 (GraphPad Software, CA, USA).

3. Result and discussion

3.1. Mucoadhesive thermosensitive in situ gel formulation of CFZ

The *in situ* ocular gels were formulated using various concentration of Pluronic® F127 and Pluronic® F68. Initially, several formulations were prepared to achieve the formulation which could form a liquid in the room temperature and change into a gel in the body temperature. It was found that the different ratio of block copolymers resulted in different gelation temperatures. The various of gelation temperature was mainly affected by the concentration of Pluronic® F127 and F68 on the gel. The gelation temperature decreased along with the increase of Pluronic® F127 and decreased in the amount of Pluronic® F68. This phenomena could explained as Pluronic® or poloxamer is generally consist of two monomer, poly-ethylene-oxide (PEO) and poly-propylene-oxide (PPO). These two monomers play an important role in affecting gelation temperature, especially PPO block [29]. A decrease of PPO block resulted in an increase of gelation temperature. Pluronic® 127 has 30% of PPO while Pluronic® F68 consist of 20% of PPO. Addition of concentration of Pluronic® F88 into the formula showed a decrease of cefazolin sol-to-gel temperature. Following our preliminary studies, the ration of 15:5 of Pluronic® F127 and Pluronic® F68 could form a gel in the body temperature, while form liquid in the room temperature. Therefore, this ratio was used in the further studies. Total of 50 g each formula was obtained, with the appearance of being clear and liquid at cold temperatures and then turning into a gel when heated (Fig. 1A and B).

It was important to determine the gelation temperature in *in situ* gel formulations in combination with or without HA as a mucoadhesive agent used in the formulation. The acceptable gelation temperatures value close to body temperature were obtained, which were in the range of 30–37 °C. The five formulations have gelation temperature values in the range of 31.3–36.7 °C. In addition, the five formulations were tested for gelation temperature again by dissolving them using STF to investigate whether the tear fluid could affect the gelation temperature of the five formulations. The five formulations that have been diluted with STF possessed gelation temperature values in the range of 30.7–36.3 °C. Based on the evaluations, desired results were obtained with the five formulations having gelation temperature values which were in the optimum gelation temperature range of 30–37 °C [10].

3.2. Mucoadhesive study of CFZ in situ gel formulation

In several years, the mucoadhesive concept has very often become a theory that has attracted a lot of attention, especially on its effectiveness in increasing the bioavailability of drugs [30]. There are many theories

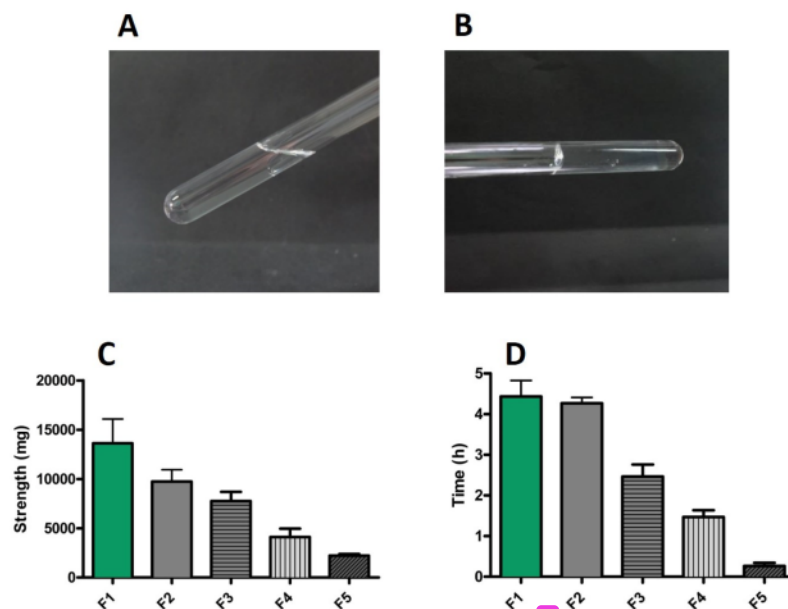


Fig. 1. CFZ mucoadhesive thermosensitive *in situ* gel formulation in room temperature (A) and CFZ mucoadhesive thermosensitive *in situ* gel formulation in body temperature (B). Mucoadhesive strength (C) and mucoadhesive time (D) of five formulations of mucoadhesive thermosensitive *in situ* gel of CFZ (mean \pm SD, n = 3).

that discuss this mucoadhesive phenomenon, one of the theories is the adsorption theory. This theory discusses the mechanism of mucoadhesion, including hydrogen bonds, van der Waals, and hydrophobic agents. Although this type is classified as weak bond, but if many bonds were formed, it could produce a strong level of adhesion [30].

In this study, there were two important aspects that were discussed in the mucoadhesive study, namely mucoadhesive strength and mucoadhesive time. Mucoadhesive strength represents the amount of weight given until the formula was separated from the tissue to observe the level of adhesion of the formulation. On the other hand, mucoadhesive time is the time required by the formulation for separating between the tissue when given some weight. The results obtained from the five formulations are depicted in Fig. 1C and D.

As can be seen in Fig. 1C and D, F1 showed the largest value in both aspects, strength and time, while F5 showed the lowest value. This was due to the variation in the concentration of mucoadhesive agents, namely HA, where F1 possessed the highest HA concentration while F5 was not mixed with HA. The value of F5 has a mucoadhesive strength value of 13625.36 ± 2474.52 mg and a mucoadhesive time of 4.43 ± 0.39 h. This value was the highest value between five formulations. This could potentially result in the increase of drug released in the eye tissue, leading to the effectiveness of the treatment. From the statistical analysis, it was also found that the *p* value was less than 0.05, indicating that HA concentrations significantly affected the mucoadhesive properties of the formulations.

3.3. pH evaluation

The five formulations had pH values in the range of 6.69–6.83 and were therefore acceptable for ophthalmic applications [31].

3.4. Viscosity of formulation at three different temperature and rheology study of gel *in situ* formulation

Polymers, however, are non-Newtonian fluids, and most often exhibit shear-thinning behavior, which is entangled, the long molecules begin to unravel and are oriented along the flow direction when the

applied deformation is high enough. Shear thinning is a property which actually makes many processing methods possible [32]. In this study, the viscosity of the thermosensitive gels was observed at 4 °C (cold

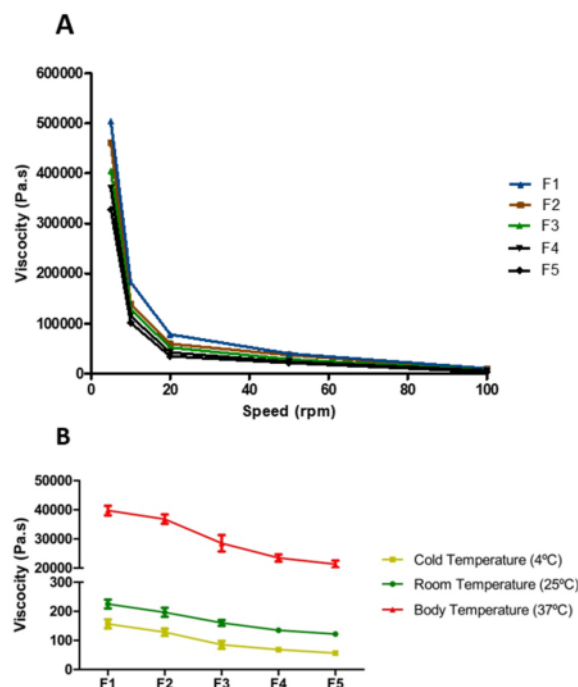


Fig. 2. Rheology of CFZ mucoadhesive-thermosensitive *in situ* gel formulation with variety of spindle rotation (rpm) (A). Viscosity of CFZ mucoadhesive-thermosensitive *in situ* gel formula in three different temperatures (B) (mean \pm SD, n = 3).

temperature), 25 °C (room temperature), and 37 °C (body temperature) (Fig. 2). Ideally, the formulation should possess the property which could form a free-flow liquid below body temperature in order to ease the administration of the formulation while changing into a semisolid preparation at temperature of the body in order to improve the contact time. With respect to the characteristic of the rheology, commonly, the gel preparation shows pseudoplastic flow. Accordingly, the thermo-sensitive formulation should be able to produce shear-thinning properties at room and body temperatures. In this type of rheological property, the viscosity of the formulation would decrease following the improvement of the shearing stress. This was due to the theory that the high velocity could influence the three-dimensional structures in the gel system, thereby reduce the low viscosity [19,29]. As shown in Fig. 2, all formulations possessed this characteristic. Accordingly, the formulations developed in this study could meet the criteria of the desired formulation. It was also found that the addition of HA as mucoadhesive agent did not influence the thermosensitive properties of the formulations.

3.5. Drug content determination of CFZ in mucoadhesive-thermosensitive *in situ* gel formulation

In an attempt to investigate whether the drug content in the formula was in agreement with the actual content, it was necessary to test the drug content. In this study, the percent of recovery drug was carried out, all formulations were determined for the percent recovery by comparing the levels of the CFZ stock solution of 10 µg/mL. It was found that the percent recovery values of the five formulations ranged from 98.33 ± 2.47% to 101.43 ± 2.60%. From these results, it can be concluded that the CFZ content in the formula was corresponded with the concentration used in the formula, and the results obtained can be accounted for the further tests.

3.6. *In vitro* drug release study

This step aimed to evaluate the amount of released drug from the thermosensitive formulations. The drug content remaining in the ocular tissue is very important for the effectiveness in the treatment of bacterial keratitis. In this study, the amount of released from mucoadhesive-thermosensitive *in situ* gel formula was compared to the solution form. It was found that in *in vitro* release study (Fig. 3), only after 3 h, the amount of released drug of control solution was 3.17 ± 0.23 mg. On the other hand, the amount of released drug in 24 h for the formulations F1, F2, F3, F4, and F5 were 1.22 ± 0.19 mg, 1.57 ± 0.14 mg, 1.70 ± 0.18 mg, 1.99 ± 0.22 mg, and 2.11 ± 0.27 mg, respectively.

From the result obtained in this study, it could be hypothesized that CFZ formulated into thermosensitive *in situ* gel was capable of controlling the release of CFZ across the dialysis membrane. As this formulation could control the release of the drug, the longer the drug in the membrane could potentially improve the effectiveness of the treatment for keratitis. It was also found that the addition of HA could reduce the drug release profiles.

It was important to investigate the release mechanism of CFZ from mucoadhesive-thermosensitive *in situ* gel formulation in *in vitro* release study. The release mechanism was performed by fitting the data result to different mathematical kinetic models with DD solver app [33]. The release mechanism was determined based on the correlation determination (R^2) value nearly to ($R^2 = 1.00$), which the correlation determination of *in vitro* study can be seen on Table 2.

Following the calculation of the mathematic models, all formula followed Higuchi's model for the release mechanism with correlation coefficient of F1, F2, F3, F4 and F4 respectively were 0,7754; 0,8189; 0,7995; 0,7909; 0,7970. The Higuchi's model indicates that the drug release in this system was gradually influenced by the swelling the matrix [34].

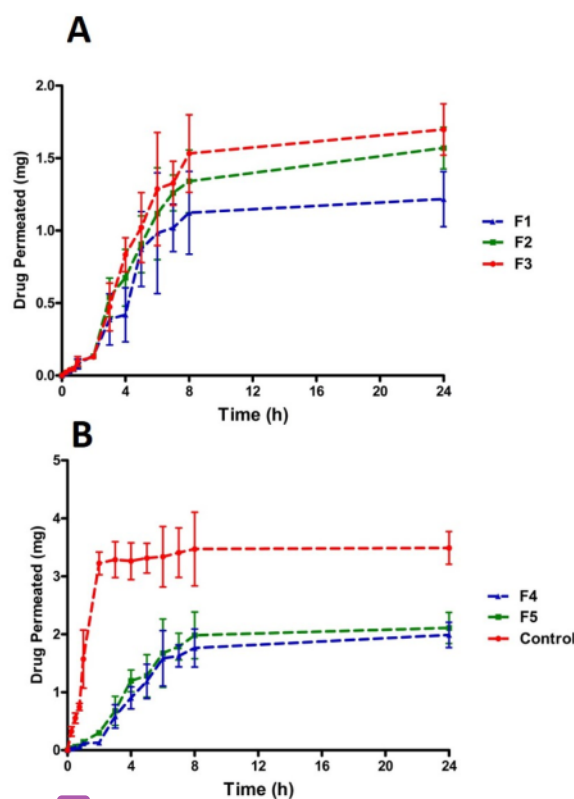


Fig. 3. *In vitro* permeation study of CFZ thermosensitive mucoadhesive *in situ* gel in dialysis membrane (mean ± SD, n = 3).

3.7. *Ex vivo* permeation study of CFZ mucoadhesive-thermosensitive *in situ* gel formulation

It is important to consider that the amount of drug concentration in the ocular tissue is very influential for the effectiveness of treatment for bacterial keratitis therapy. In this case, the drug was permeated through the *ex vivo* cornea tissue and the results are depicted in Fig. 4A. From the data, it was shown that after 24 h, control solution could produce 3.85 ± 0.37 mg of permeation amount. Furthermore, in the case of thermosensitive *in situ* gel, the amount of CFZ permeating after 24 h was significantly lower ($p < 0.05$) compared to control solution which was in the range between 1.01 ± 0.11 mg to 1.80 ± 0.21 mg. In agreement with *in vitro* results, the mathematic model of the *ex vivo* permeation also followed Higuchi's model with determination coefficient was 0,7754; 0,8189; 0,7995; 0,7909; and 0,7970 respectively (Table 3). Moreover, flux of permeation (J_{ss}) and permeation coefficient (K_p) of CFZ from mucoadhesive-thermosensitive were calculated and compared to the control solution (Table 4). It was found that the J_{ss} and K_p values of control solution was significantly higher ($p < 0.05$) compared to thermosensitive formulations. Accordingly, considering the values and kinetic model results, similar to *in vitro* drug release study, this study depicted that the incorporation of CFZ into thermogelling system could control the release the permeation through the ocular tissue.

For the further analysis, the *ex vivo* retention was measured. The *ex vivo* retention was carried out to observe the amount of drug content localized in the corneal tissue which could be used to estimate the effectiveness of this approach [11]. This was performed by calculating the concentration level retained in the tissue after 1, 8, and 24 h of the application of each formulation. The retention results were also

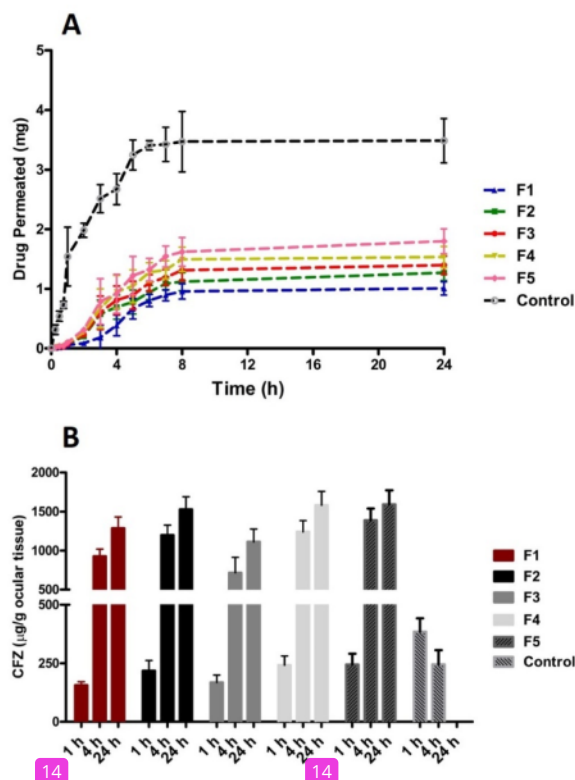


Fig. 4. Ex vivo permeation study of CFZ mucoadhesive thermosensitive *in situ* gel in ocular tissue compared with control eye drop (A). Ex vivo retention of mucoadhesive thermosensitive *in situ* gel and control eye drop of CFZ in ocular tissue (B) (mean \pm SD, n = 3).

Table 2
In vitro correlation coefficient of CFZ from mucoadhesive-thermosensitive *in situ* gel formulation in different mathematic kinetic models.

Mathematic model	Correlation Determination (R^2)				
	F1	F2	F3	F4	F5
Zero Order Kinetics	0,5930	0,6514	0,6274	0,6187	0,5935
First Order Kinetics	0,4387	0,5148	0,4903	0,4842	0,4100
Higuchi Models	0,7754	0,8189	0,7995	0,7909	0,7970
Krossmeyer – Peppas Model	0,2506	-0,5205	0,2146	0,2096	-0,4888
Hixson – Crowell Model	0,4676	0,3980	0,3699	0,3217	0,3891

Table 3
Ex vivo determination coefficient of CFZ from mucoadhesive-thermosensitive.

Mathematic model	Correlation Coefficient (R^2)				
	F1	F2	F3	F4	F5
Zero Order Kinetics	0,5930	0,6514	0,6274	0,6187	0,5935
First Order Kinetics	0,4387	0,5148	0,4903	0,4842	0,4100
Higuchi Models	0,7754	0,8189	0,7995	0,7909	0,7970
Krossmeyer – Peppas Model	0,2506	-0,5205	0,2146	0,2096	-0,4888
Hixson – Crowell Model	0,4676	0,3980	0,3699	0,3217	0,3891

compared to control solution and the results are exhibited in Fig. 4B.

According the bar charts, it was shown that the incorporation CFZ to the thermosensitive hydrogel could increase the location of CFZ in

Table 4
Flux of permeation (Jss) and permeation coefficient (Kp) of CFZ from mucoadhesive-thermosensitive and control solution (mean \pm SD, n = 3).

Formulation	Jss ($\mu\text{g}/(\text{cm}^2\text{h})$)	Kp (cm/h)
F1	127.23 \pm 11.98	0.054 \pm 0.001
F2	133.93 \pm 10.39	0.065 \pm 0.001
F3	149.11 \pm 12.13	0.078 \pm 0.002
F4	154.17 \pm 13.09	0.087 \pm 0.002
F5	176.27 \pm 16.12	0.093 \pm 0.001
Control solution	687.23 \pm 41.23	0.233 \pm 0.01

ocular tissue. The amount of CFZ localized in the ocular tissue increased from 1 h to 24 h after the application of the formulation. After 24 h, the amount of CFZ detected was in the range of 1287.11 \pm 143.72 $\mu\text{g}/\text{g}$ – 1592.19 \pm 179.34 $\mu\text{g}/\text{g}$. Interestingly, no CFZ was detected in the case of control solution after 24 h of application. Therefore, the thermosensitive formulation could not only control the release of CFZ, but also improve the concentration of CFZ in the ocular tissue, which could potentially improve its effectiveness in the treatment of bacterial keratitis.

3.8. Hemolysis study

It was crucial to investigate the potential toxicity of the new formulation. In this study, we used hemolysis parameter of the blood red cells as initial evaluation to investigate the potential toxicity which could be caused by the formulations. Several studies have used this evaluation to screen the potential of toxicity of numerous drug delivery systems [20,26,35,36]. The results of this study are exhibited in Fig. 5A and B, showing that the hemolysis percentages were below 1.5% in all concentrations tested. Accordingly, the formulations developed in this study were considerably safe.

3.9. In vitro antibacterial activity

It was critical to ensure that the incorporation of CFZ into thermosensitive *in situ* gel did not reduce its antimicrobial activity. In this study, we evaluated the antimicrobial activity using agar diffusion method against *Pseudomonas aeruginosa*. Fig. 5C depicts the representative image of this study. It was found that the inhibition zones of CFZ solution and CFZ in the thermosensitive *in situ* gel were 21.98 \pm 1.98 mm and 20.76 \pm 1.21 mm. Analyzed statistically, there was no significant difference ($p > 0.05$) between those values, indicating that the formulation did not affect the antimicrobial activity of CFZ against *Pseudomonas aeruginosa*.

3.10. In vivo irritation evaluation

We further assess the irritation of our approach *in vivo* in rabbits. Fig. 6 shows the result of the treatment after 4 days. The rabbit eye with CFZ *in situ* gel showed a comparable result compared to the rabbit eye without treatment and the administration of NaCl 0.9%. On the other hand, following the administration of SLS, the rabbit's eyes were observed to be red and the ciliary muscles were swollen. Therefore, the approach developed in this study were not found to be irritable to the ocular tissue following the multiple day application.

3.11. Histopathological evaluation

Following the irritation evaluation, we collected the ocular tissues and observed using histopathological examinations. The results of this study are depicted in Fig. 7. It was found that in the administration of the formulation, NaCl 0.9% and no treatment groups, no infiltration, congestion and edema were observed. In contrast, infiltration, congestion and edema were all observed after the administration of SLS. Therefore, the thermosensitive formulations were potentially safe for ocular administration.

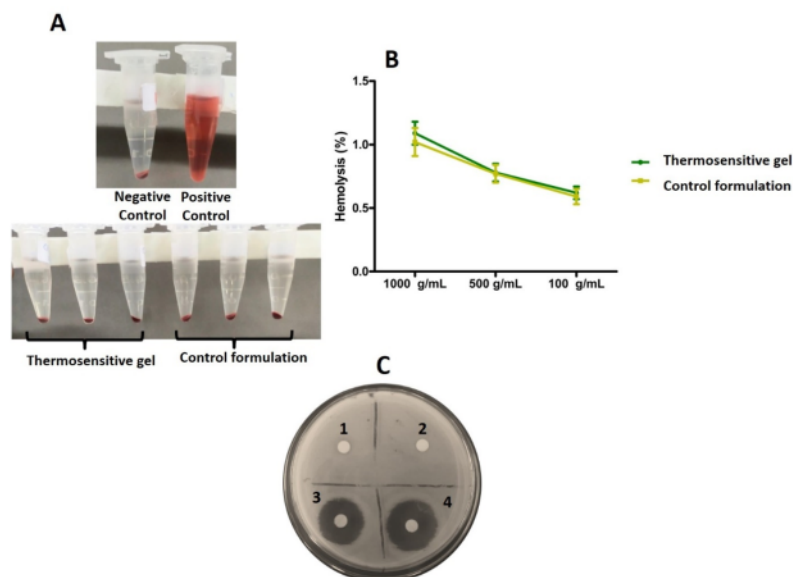


Fig. 5. Representative images of *in vitro* hemolysis study in red blood cells (A). Hemolysis percentages of thermosensitive and control formulations (B) (mean \pm SD, n = 3). Inhibition zones of blank disc (1), water (2), CFZ solution (3) and CFZ thermosensitive formulation against *Pseudomonas aeruginosa* (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

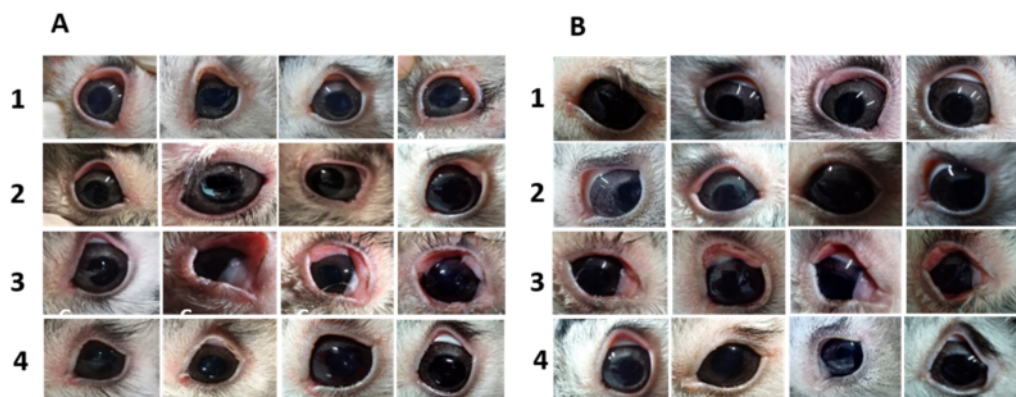


Fig. 6. Rabbit's eye on day one treatment (A) and last day treatment (B), (1 = with CFZ), (B = With NaCl 0,9%), (C = with Sodium lauryl sulfate (SLS)), (D = without treatment).

Overall outcomes have proven that the incorporation of CFZ into thermosensitive-mucoadhesive hydrogel could potentially improve the localization of CFZ in the ocular tissue, while controlling the release behavior. This approach could potentially be beneficial in the treatment of bacterial keratitis as it was also found that no irritations were observed following the multiple day applications, and importantly, no potential toxicity was found. The use of thermosensitive hydrogel in ocular drug delivery have been widely explored. However, several aspects should be considered. As this was intended to ocular administration, before moving to the industrial steps, sterilization process should be developed. Several studies have shown that Pluronic®-based hydrogels could be sterilized using steam heat sterilization with autoclave [37,38]. However, due to the instability of CFZ in high temperature, gamma radiation could be the appropriate method for the sterilization. The development of this system is still in the early step. Therefore, it is also crucial to further optimize the formulation to ensure that the hydrogel

possess adequate properties after the administration to the patients. As the formulation is in the liquid form, the hydrogel could be administered using the same method as the eye drops. Additionally, the information regarding the transformation of the liquid to the gel after the administration should be given to the patients. However, prior to the application in the clinic, further study investigating *in vivo* pharmacokinetic and pharmacodynamic studies in appropriate animal models should now be conducted.

4. Conclusion

Based on all evaluation results of the mucoadhesive-thermosensitive *in situ* gel of CFZ, it can be concluded that the CFZ *in situ* gel preparation could increase the contact time with the eye up to 4 h. The formulations were able to sustain the release of CFZ and improve the retention in the eye tissue when compared to control solution with no

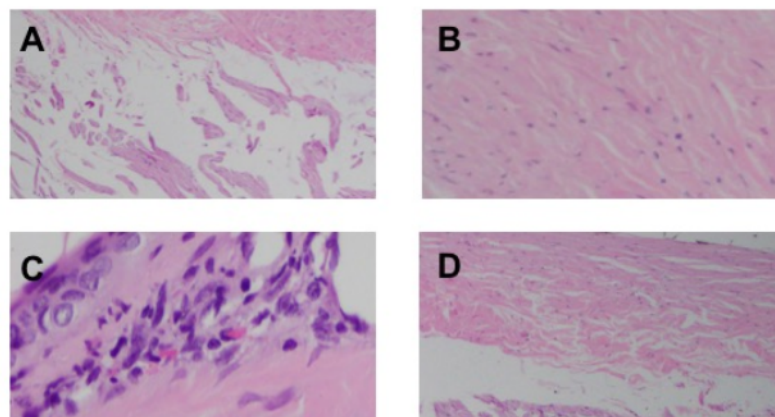


Fig. 7. Histopathological result of (A) NaCl 0,9% solution; (B) CFZ mucoadhesive-thermosensitive *in situ* gel formula; (C) sodium lauryl sulfate (SLS) solution; and (D) without treatment.

significant change in antimicrobial activity. Following the hemolysis assay, the formulation was potentially safe. Importantly, the *in situ* gel preparation also did not irritate the experimental animals applied with the *in situ* gel preparation.

Author contributions

Muh. Al Fiqri: Conceptualization, Methodology, Funding acquisition, Writing – original draft. **Alhidayah:** Methodology, Writing – original draft. **Nirmayanti:** Methodology, Writing – original draft. **Ummu Athiyah:** Methodology, Data curation. **Patricia Layadi:** Methodology, Data curation. **Tamara Gabriela Angeleve Fadjar:** Data curation, Validation **Andi Dian Permana:** Conceptualization, Project administration, Funding acquisition, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

This study was fully funded by Student Creativity Program (PKM), Directorate General of Higher Education, Ministry of Education and Culture of Indonesia. The authors also wish to thank to BASF Indonesia for providing Pluronic®.

References

- [1] D. Tena, N. Rodríguez, L. Toribio, A. González-Praetorius, Infectious keratitis: microbiological review of 297 cases, *Jpn. J. Infect. Dis.* 72 (2019) 121–123.
- [2] F.U. Musa, R. Tailor, A. Gao, E. Hutley, S. Rauz, R.A.H. Scott, Contact lens-related microbial keratitis in deployed British military personnel, *Br. J. Ophthalmol.* 94 (2010) 988–993.
- [3] S. Egrilmez, Ş. Yıldırım-Theveny, Treatment-resistant bacterial keratitis: challenges and solutions, *Clin. Ophthalmol.* 14 (2020) 287–297.
- [4] A. Austin, T. Lietman, J. Rose-Nussbaumer, Update on the management of infectious keratitis, *Ophthalmology* 124 (2017) 1678–1689, <https://doi.org/10.1016/j.ophtha.2017.05.012>.
- [5] U.M. Güven, M.S. Berkman, B. Şenel, Y. Yazan, Development and *in vitro/in vivo* evaluation of thermo-sensitive *in situ* gelling systems for ocular allergy, *Brazilian J. Pharm. Sci.* 55 (2019) 1–11, <https://doi.org/10.1590/s2175-97902019000117511>.

- [6] N. Üstündağ Okur, E.Ş. Çağlar, P.I. Siafaka, Novel ocular drug delivery systems: an update on microemulsions, *J. Ocul. Pharmacol. Therapeut.* 36 (2020) 342–354.
- [7] M.F. Rozi, A.S. Mohamad Sabere, Review on conventional and novel topical ocular drug delivery system, *J. Pharm. (Lahore)* 1 (2021) 19–26.
- [8] S.A. Young, H. Riahi-zhad, B.G. Amsden, In situ -forming, mechanically resilient hydrogels for cell delivery, *J. Mater. Chem. B* 7 (2019) 5742–5761, <https://doi.org/10.1039/c9tb01398a>.
- [9] T. Irimia, C.E. Dinu-Privu, M.V. Ghica, D. Lupulescu, D.L. Muntean, D.I. Udeanu, L. Popa, Chitosan-based *in situ* gels for ocular delivery of therapeutics: a state-of-the-art review, *Mar. Drugs* 16 (2018).
- [10] B. Vignani, S. Rossi, G. Sandri, M.C. Bonferoni, C.M. Caramella, F. Ferrari, Recent advances in the development of *in situ* gelling drug delivery systems for non-parenteral administration routes, *Pharmaceutics* 12 (2020) 1–29.
- [11] D. Jain, V. Kumar, S. Singh, A. Mullertz, D. Bar-Shalom, Newer trends in *in situ* gelling systems for controlled ocular drug delivery, *J. Anal. Pharm. Res.* 2 (2016) 1–16, <https://doi.org/10.15406/japlr.2016.02.00022>.
- [12] Z.M.A. Fathalla, A. Vangala, M. Longman, K.A. Khaled, A.K. Hussein, O.H. El-Garhy, R.G. Alany, Poloxamer-based thermoresponsive ketorolac tromethamine *in situ* gel preparations: design, characterisation, toxicity and transcorneal permeation studies, *Eur. J. Pharm. Biopharm.* 114 (2017) 119–134.
- [13] K.A. Soliman, K. Ullah, A. Shah, D.S. Jones, T.R.R. Singh, Poloxamer-based *in situ* gelling thermoresponsive systems for ocular drug delivery applications, *Drug Discov. Today* 24 (2019) 1575–1586.
- [14] K. Zhang, X. Shi, X. Lin, C. Yao, L. Shen, Y. Feng, Poloxamer-based *in situ* hydrogels for controlled delivery of hydrophilic macromolecules after intramuscular injection in rats, *Drug Deliv.* 22 (2015) 375–382.
- [15] F. Zahir-Jouzani, J.D. Wolf, F. Atyabi, A. Bernkop-Schnürch, *In situ* gelling and mucoadhesive polymers: why do they need each other? *Expet Opin. Drug Deliv.* 15 (2018) 1007–1019.
- [16] S. Cordeiro, B. Silva, A.M. Martins, H.M. Ribeiro, L. Gonçalves, J. Marto, Antioxidant-loaded mucoadhesive nanoparticles for eye drug delivery: a new strategy to reduce oxidative stress, *Processes* 9 (2021) 1–19.
- [17] M.K. Prinsen, C.F.M. Hendriksen, C.A.M. Krul, R.A. Woutersen, The Isolated Chicken Eye test to replace the Draize test in rabbits, *Regul. Toxicol. Pharmacol.* 85 (2017) 132–149, <https://doi.org/10.1016/j.yrtph.2017.01.009>.
- [18] A. Khattab, S. Marzok, M. Ibrahim, 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.* 53 (2019), 101134, <https://doi.org/10.1016/j.jddst.2019.101134>.
- [19] A.D. Permana, R. Nurul, P. Layadi, A. Himawan, N. Juniarti, Q. Kurnia, E. Utomo, S. Aulia, A. Arjuna, R.F. Donnelly, Thermosensitive and mucoadhesive *in situ* ocular gel for effective local delivery and antifungal activity of itraconazole nanocrystal in the treatment of fungal keratitis, *Int. J. Pharm.* 602 (2021), 120623.
- [20] C.K. Enggi, H.T. Isa, S. Sulistiawati, K.A.R. Ardika, S. Wijaya, R.M. Asri, S. A. Mardikasari, R.F. Donnelly, A.D. Permana, Development of thermosensitive and mucoadhesive gels of cabotegravir for enhanced permeation and retention profiles in vaginal tissue: a proof of concept study, *Int. J. Pharm.* 609 (2021), 121182, <https://doi.org/10.1016/j.ijpharm.2021.121182>.
- [21] Alhidayah, M. Al Fiqri, Nirmayanti, U. Athiyah, T.G.A. Fadjar, F. Nainu, A. Arjuna, A.D. Permana, Validation of spectrophotometric method for quantification of cefazoline in simulated tear fluid and porcine ocular tissue from thermosensitive-mucoadhesive *in situ* ocular gel preparation, *Chem. Data Collect.* 36 (2021), 100793, <https://doi.org/10.1016/j.cdc.2021.100793>.
- [22] A.D. Permana, R.N. Utami, A.J. Courtenay, M.A. Manggau, R.F. Donnelly, L. Rahman, Phytosomal nanocarriers as platforms for improved delivery of natural antioxidant and photoprotective compounds in propolis: an approach for enhanced both dissolution behaviour in biorelevant media and skin retention profiles, *J. Photochem. Photobiol. B Biol.* 205 (2020), 111846.

- [23] B. Silva, J. Marto, B.S. Braz, E. Delgado, A.J. Almeida, L. Gonçalves, New nanoparticles for topical ocular delivery of erythropoietin, *Int. J. Pharm.* 576 (2020), 119020, <https://doi.org/10.1016/j.ijpharm.2020.119020>.
- [24] M.M. Silva, R. Calado, J. Marto, A. Bettencourt, J. Almeida, L.M.D. Gonçalves, Chitosan Nanoparticles as a Mucoadhesive Drug Delivery System for Ocular Administration, 2017, pp. 1–16, <https://doi.org/10.3390/md15120370>.
- [25] S.L.J. Blok, G.E. Engels, W. van Oeveren, In vitro hemocompatibility testing: the importance of fresh blood, *Biointerphases* 11 (2016), 029802, <https://doi.org/10.1116/1.4941850>.
- [26] P.W.R. Ananda, D. Elim, H.S. Zaman, W. Muslimin, M.G.R. Tunggeng, A. D. Permana, Combination of transdermal patches and solid microneedles for improved transdermal delivery of primaquine, *Int. J. Pharm.* 609 (2021), 121204, <https://doi.org/10.1016/j.ijpharm.2021.121204>.
- [27] M. Lee, J.H. Hwang, K.M. Lim, Alternatives to in vivo Draize rabbit eye and skin irritation tests with a focus on 3D reconstructed human cornea-like epithelium and epidermis models, *Toxicol. Res.* 33 (2017) 191–203.
- [28] P. Kalra, L.K. Ahirwar, R. Mittal, K. Ranjith, S. Das, K. Manjulatha, B. Bagga, A. Mohamed, J. Joseph, S. Sharma, Clinical and histopathological evaluation of a rabbit model for pythium insidiosum keratitis, *Curr. Eye Res.* 45 (2020) 542–549.
- [29] A.D. Permana, E. Utomo, M.R. Pratama, M.N. Amir, Q.K. Anjani, S.A. Mardikasari, S. Sumarheni, A. Himawan, A. Arjuna, U. Usmanengsi, R.F. Donnelly, Bioadhesive-thermosensitive in situ vaginal gel of the gel flake-solid dispersion of itraconazole for enhanced antifungal activity in the treatment of vaginal candidiasis, *ACS Appl. Mater. Interfaces* 13 (2021) 18128–18141, <https://doi.org/10.1021/acsmi.1c03422>.
- [30] P. Subramanian, Mucoadhesive delivery system: a smart way to improve bioavailability of nutraceuticals, *Foods* 10 (2021).
- [31] M. Dhaval, J. Devani, R. Parmar, M.M. Soniwala, J. Chavda, Formulation and optimization of microemulsion based sparfloxacin in-situ gel for ocular delivery: in vitro and ex vivo characterization, *J. Drug Deliv. Sci. Technol.* 55 (2020), 101373.
- [32] J. Aho, J.P. Boetker, S. Baldursdottir, J. Rantanen, Rheology as a tool for evaluation of melt processability of innovative dosage forms, *Int. J. Pharm.* 494 (2015) 623–642.
- [33] J. Zuo, Y. Gao, N. Bou-Chacra, R. Löbenberg, Evaluation of the DDSolver software applications, *BioMed Res. Int.* 2014 (2014), <https://doi.org/10.1155/2014/204925>.
- [34] A.D. Permana, M.T.C. McCrudden, R.F. Donnelly, Enhanced intradermal delivery of nanosuspensions of antifilaria drugs using dissolving microneedles: a proof of concept study, *Pharmaceutics* 11 (2019) 346.
- [35] H.A. Qonita, N. Syafika, V. Valensie, J. Kamba, A. Maulana, A.D. Permana, Development water in oil nanoemulsion of diethylcarbamazine for enhanced the characteristics for lymphatic targeting: a proof of concept study, *J. Indian Chem. Soc.* 99 (2022), 100395, <https://doi.org/10.1016/j.jics.2022.100395>.
- [36] M. Mir, A.D. Permana, N. Ahmed, G.M. Khan, A. ur Rehman, R.F. Donnelly, Enhancement in site-specific delivery of carvacrol for potential treatment of infected wounds using infection responsive nanoparticles loaded into dissolving microneedles: a proof of concept study, *Eur. J. Pharm. Biopharm.* 147 (2020) 57–68.
- [37] D. Rafael, F. Andrade, F. Martinez-Trucharte, J. Basas, J. Seras-Franzoso, M. Palau, X. Gomis, M. Pérez-Burgos, A. Blanco, A. López-Fernández, R. Vélez, I. Abasolo, M. Aguirre, J. Gavaldà, S. Schwartz, Sterilization procedure for temperature-sensitive hydrogels loaded with silver nanoparticles for clinical applications, *Nanomaterials* 9 (2019), <https://doi.org/10.3390/nano9030380>.
- [38] M. Raval, H. Bagada, Formulation and evaluation of cyclodextrin-based thermosensitive in situ gel of azithromycin for periodontal delivery, *J. Pharm. Innov.* 16 (2021) 67–84, <https://doi.org/10.1007/s12247-019-09422-3>.

ORIGINALITY REPORT

21 %
SIMILARITY INDEX

13 %
INTERNET SOURCES

18 %
PUBLICATIONS

3 %
STUDENT PAPERS

PRIMARY SOURCES

1 Andi Dian Permana, Emilia Utomo, Muhammad Rezky Pratama, Muh. Nur Amir et al. " Bioadhesive-Thermosensitive Vaginal Gel of the Gel Flake-Solid Dispersion of Itraconazole for Enhanced Antifungal Activity in the Treatment of Vaginal Candidiasis ", ACS Applied Materials & Interfaces, 2021
Publication **2** %

2 Abeer Khattab, Sarah Marzok, Magdy Ibrahim. "Development of optimized mucoadhesive thermosensitive pluronic based in situ gel for controlled delivery of Latanoprost: Antiglaucoma efficacy and stability approaches", Journal of Drug Delivery Science and Technology, 2019
Publication **1** %

3 Ardiyah Nurul Fitri Marzaman, Sartini, Mukarram Mudjahid, Tri Puspita Roska, Anwar Sam, Andi Dian Permana. "Development of chloramphenicol whey protein-based microparticles incorporated into thermoresponsive in situ hydrogels for **1** %

improved wound healing treatment",
International Journal of Pharmaceutics, 2022

Publication

4	www.mdpi.com Internet Source	1 %
5	Aho, Johanna, Johan P. Boetker, Stefania Baldursdottir, and Jukka Rantanen. "Rheology as a tool for evaluation of melt processability of innovative dosage forms", International Journal of Pharmaceutics, 2015. Publication	1 %
6	Pooja Jain, Chandra Prakash Jaiswal, Mohd. Aamir Mirza, Md. Khalid Anwer, Zeenat Iqbal. " Preparation of levofloxacin loaded gel for sustained ocular delivery: and evaluations ", Drug Development and Industrial Pharmacy, 2019 Publication	1 %
7	www.researchsquare.com Internet Source	1 %
8	tel.archives-ouvertes.fr Internet Source	1 %
9	Nirmayanti Nirmayanti, Alhidayah Alhidayah, Jessica Theodor Usman, Julika Fajrika Nur, Muh. Nur Amir, Andi Dian Permana. "Combinatorial Approach of Thermosensitive Hydrogels and Solid Microneedles to Improve	<1 %

Transdermal Delivery of Valsartan: an In Vivo Proof of Concept Study", AAPS PharmSciTech, 2022

Publication

10

[medcraveonline.com](https://www.medcraveonline.com)

Internet Source

<1 %

11

www.frontiersin.org

Internet Source

<1 %

12

unsworks.unsw.edu.au

Internet Source

<1 %

13

one.aao.org

Internet Source

<1 %

14

Dalia M.N. Abouhoussein, Abeer Khattab, Noha A. Bayoumi, Ashgan F. Mahmoud, Tamer M. Sakr. "Brain targeted rivastigmine mucoadhesive thermosensitive In situ gel: Optimization, in vitro evaluation, radiolabeling, in vivo pharmacokinetics and biodistribution", Journal of Drug Delivery Science and Technology, 2018

Publication

<1 %

15

biointerfaceresearch.com

Internet Source

<1 %

16

"Bioadhesives in Drug Delivery", Wiley, 2020

Publication

<1 %

17 Submitted to Badan PPSDM Kesehatan
Kementerian Kesehatan <1 %
Student Paper

18 Kayoko Hayashida, Taishi Higashi, Daichi
Kono, Keiichi Motoyama, Koki Wada,
Hidetoshi Arima. "Preparation and evaluation
of cyclodextrin polypseudorotaxane with
PEGylated liposome as a sustained release
drug carrier", Beilstein Journal of Organic
Chemistry, 2014 <1 %
Publication

19 fanlab.bme.umich.edu <1 %
Internet Source

20 Miri Lee, Jee-Hyun Hwang, Kyung-Min Lim. "
Alternatives to Draize Rabbit Eye and Skin
Irritation Tests with a Focus on 3D
Reconstructed Human Cornea-Like Epithelium
and Epidermis Models ", Toxicological
Research, 2017 <1 %
Publication

21 res.mdpi.com <1 %
Internet Source

22 turkjps.org <1 %
Internet Source

23 www.ncbi.nlm.nih.gov <1 %
Internet Source

24	ore.exeter.ac.uk Internet Source	<1 %
25	repository.unhas.ac.id Internet Source	<1 %
26	Ripal Gaudana, Hari Krishna Ananthula, Ashwin Parenky, Ashim K. Mitra. "Ocular Drug Delivery", The AAPS Journal, 2010 Publication	<1 %
27	gacetasanitaria.org Internet Source	<1 %
28	storage.googleapis.com Internet Source	<1 %
29	eprints.lmu.edu.ng Internet Source	<1 %
30	www.researchgate.net Internet Source	<1 %
31	Andi Dian Permana, Sarah A. Stewart, Juan Domínguez-Robles, Muh. Nur Amir, Muh. Akbar Bahar, Ryan F. Donnelly, Eneko Larraneta. "Development and validation of a high-performance liquid chromatography method for levothyroxine sodium quantification in plasma for pre-clinical evaluation of long-acting drug delivery systems", Analytical Methods, 2021 Publication	<1 %

32 Dharmendra Jain. "Newer Trends in In Situ Gelling Systems for Controlled Ocular Drug Delivery", Journal of Analytical & Pharmaceutical Research, 2016

Publication

<1 %

33 weitzlab.seas.harvard.edu

Internet Source

<1 %

34 Submitted to Universiti Malaysia Terengganu UMT

Student Paper

<1 %

35 Furqan A. Maulvi, Kiran H. Shetty, Ditixa T. Desai, Dinesh O. Shah, Mark D.P. Willcox. "Recent advances in ophthalmic preparations: Ocular barriers, dosage forms and routes of administration", International Journal of Pharmaceutics, 2021

Publication

<1 %

36 Kapil khatri, Shikha Jain, Satish Shilpi. "Nasal In-situ Gel: An Approach to Enhance Therapeutic Benefits of the Drug", Drug Delivery Letters, 2020

Publication

<1 %

37 Submitted to Queen's University of Belfast

Student Paper

<1 %

38 Beatriz Silva, Joana Marto, Berta São Braz, Esmeralda Delgado, António José Almeida, Lídia Gonçalves. "New nanoparticles for

<1 %

topical ocular delivery of erythropoietin",
International Journal of Pharmaceutics, 2020

Publication

39

Insan Sunan Kurniawansyah, Taofik Rusdiana, Iyan Sopyan, Handrian Ramoko, Habibah A. Wahab, Anas Subarnas. "In situ ophthalmic gel forming systems of poloxamer 407 and hydroxypropyl methyl cellulose mixtures for sustained ocular delivery of chloramphenicol: optimization study by factorial design", Heliyon, 2020

Publication

<1 %

40

www.hindawi.com

Internet Source

<1 %

41

www.tandfonline.com

Internet Source

<1 %

42

Teklit Gebregiorgis Amabye. "Phytochemical and Antibacterial Activity of Moringa Oleifera Available in the Market of Mekelle", Journal of Analytical & Pharmaceutical Research, 2016

Publication

<1 %

43

Xuejing Cheng, Huiyu Chen, Fang Yang, Jiayu Hong, Yiyun Cheng, Jingjing Hu. "All-small-molecule supramolecular hydrogels assembled from guanosine 5'-monophosphate disodium salt and tobramycin for the treatment of bacterial keratitis", Bioactive Materials, 2022

<1 %

44

dspace.tbzmed.ac.ir:8080

Internet Source

<1 %

45

mdpi-res.com

Internet Source

<1 %

46

uhra.herts.ac.uk

Internet Source

<1 %

47

www.dovepress.com

Internet Source

<1 %

48

Andi Dian Permana, Alejandro J. Paredes, Fabiana Volpe Zanutto, Muh. Nur Amir et al. "Albendazole Nanocrystal-Based Dissolving Microneedles with Improved Pharmacokinetic Performance for Enhanced Treatment of Cystic Echinococcosis", ACS Applied Materials & Interfaces, 2021

Publication

<1 %

49

Archana S. Pokkalath, Apurva Sawant, Sujata P. Sawarkar. "Herbal medicine for ocular diseases: An age old therapy and its future perspective", Journal of Drug Delivery Science and Technology, 2022

Publication

<1 %

50

Lijie Wang, Hao Pan, Donghao Gu, Pingfei Li, Yupei Su, Weisan Pan. "A composite system combining self-targeted carbon dots and thermosensitive hydrogels for challenging

<1 %

ocular drug delivery", Journal of
Pharmaceutical Sciences, 2021

Publication

51

Pinal Chaudhari, Ranjitha Naik, Lakshmi Sruthi Mallela, Sanhita Roy et al. "A Supramolecular Thermosensitive Gel of Ketoconazole for Ocular Applications: In Silico, In Vitro, and Ex Vivo Studies", International Journal of Pharmaceutics, 2021

Publication

<1 %

52

prp.hec.gov.pk

Internet Source

<1 %

53

pubs.rsc.org

Internet Source

<1 %

54

www.investigo.biblioteca.uvigo.es

Internet Source

<1 %

55

www.molbiolcell.org

Internet Source

<1 %

56

www.revistas.usp.br

Internet Source

<1 %

57

Amarjitsing Rajput, Aditi Bariya, Ahmed Allam, Sarah Othman, Shital B. Butani. "In situ nanostructured hydrogel of resveratrol for brain targeting: in vitro-in vivo characterization", Drug Delivery and Translational Research, 2018

Publication

<1 %

58

Anindita Chowhan, Tapan Kumar Giri.
"Polysaccharide as renewable responsive biopolymer for in situ gel in the delivery of drug through ocular route", International Journal of Biological Macromolecules, 2020
Publication

<1 %

59

Himanshu Gupta, Sanyog Jain, Rashi Mathur, Pushpa Mishra, Anil K. Mishra, T. Velpandian.
"Sustained Ocular Drug Delivery from a Temperature and pH Triggered Novel In Situ Gel System", Drug Delivery, 2008
Publication

<1 %

60

José L. Soriano-Ruiz, Joaquim Suñer-Carbó, Ana C. Calpena-Campmany, Nuria Bozal-de Febrer et al. "Clotrimazole multiple W/O/W emulsion as anticandidal agent: Characterization and evaluation on skin and mucosae", Colloids and Surfaces B: Biointerfaces, 2019
Publication

<1 %

61

Mariana Silva, Raquel Calado, Joana Marto, Ana Bettencourt, António Almeida, Lídia Gonçalves. "Chitosan Nanoparticles as a Mucoadhesive Drug Delivery System for Ocular Administration", Marine Drugs, 2017
Publication

<1 %

62

Sohail Akhter. "Investigation of Nanoemulsion System for Transdermal Delivery of

<1 %

Domperidone: Ex-vivo and in vivo Studies",
Current Nanoscience, 11/01/2008

Publication

63

innovareacademics.in

Internet Source

<1 %

64

link.springer.com

Internet Source

<1 %

65

pubmed.ncbi.nlm.nih.gov

Internet Source

<1 %

66

pure.qub.ac.uk

Internet Source

<1 %

67

Lalit Kishore Ahirwar, Paavan Kalra, Savitri Sharma, Ashik Mohamed, Ruchi Mittal, Sujata Das, Bhupesh Bagga. "Linezolid shows high safety and efficacy in the treatment of Pythium insidiosum keratitis in a rabbit model", Experimental Eye Research, 2021

Publication

<1 %

68

Sahreena Lakhundi, Ruqaiyyah Siddiqui, Naveed Ahmed Khan. "Pathogenesis of microbial keratitis", Microbial Pathogenesis, 2017

Publication

<1 %

69

"Surface Modification of Nanoparticles for Targeted Drug Delivery", Springer Science and Business Media LLC, 2019

Publication

<1 %

70

Andi Dian Permana, Rifka Nurul Utami, Aaron J. Courtenay, Marianti A. Manggau, Ryan F. Donnelly, Latifah Rahman. "Phytosomal nanocarriers as platforms for improved delivery of natural antioxidant and photoprotective compounds in propolis: An approach for enhanced both dissolution behaviour in biorelevant media and skin retention profiles", *Journal of Photochemistry and Photobiology B: Biology*, 2020

Publication

<1 %

71

Annamaria Cucina, Samira Filali, Arnaud Risler, Camille Febvay et al. "Dual 0.02% chlorhexidine digluconate – 0.1% disodium EDTA loaded thermosensitive ocular gel for *Acanthamoeba* keratitis treatment", *International Journal of Pharmaceutics*, 2019

Publication

<1 %

72

Sankalia, M.G.. "Reversed chitosan-alginate polyelectrolyte complex for stability improvement of alpha-amylase: Optimization and physicochemical characterization", *European Journal of Pharmaceutics and Biopharmaceutics*, 200702

Publication

<1 %

73

Sara S. Barakat, Maha Nasr, Rania F. Ahmed, Sabry S. Badawy, Samar Mansour. "Intranasally administered in situ gelling

<1 %

nanocomposite system of dimenhydrinate:
preparation, characterization and
pharmacodynamic applicability in
chemotherapy induced emesis model",
Scientific Reports, 2017

Publication

Exclude quotes On

Exclude matches < 5 words

Exclude bibliography On