# BUKTI SUBMIT MANUSKRIP

Universitas Hasanuddin

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

# Submission to Journal of Drug Delivery Science and Technology - manuscript number

1 message

**Journal of Drug Delivery Science and Technology** <em@editorialmanager.com> Mon, Jul 11, 2022 at 9:20 PM

Reply-To: Journal of Drug Delivery Science and Technology <support@elsevier.com>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

*This is an automated message.*

Manuscript Number: JDDST-D-22-01553
Enhanced localization of cefazoline sodium in the ocular tissue using thermosensitive-mucoadhesive hydrogels: Formulation development, hemocompatibility and in vivo irritation studies

Dear Dr. Permana,

Your above referenced submission has been assigned a manuscript number: JDDST-D-22-01553.

To track the status of your manuscript, please log in as an author at https://www.editorialmanager.com/jddst/, and navigate to the "Submissions Being Processed" folder.

Thank you for submitting your work to this journal.

Kind regards,
Journal of Drug Delivery Science and Technology

More information and support

You will find information relevant for you as an author on Elsevier's Author Hub: https://www.elsevier.com/authors

FAQ: How can I reset a forgotten password?
https://service.elsevier.com/app/answers/detail/a_id/28452/supporthub/publishing/
For further assistance, please visit our customer service site: https://service.elsevier.com/app/home/supporthub/publishing/
Here you can search for solutions on a range of topics, find answers to frequently asked questions, and learn more about Editorial Manager via interactive tutorials. You can also talk 24/7 to our customer support team by phone and 24/7 by live chat and email

#AU_JDDST#

To ensure this email reaches the intended recipient, please do not delete the above code

# BUKTI REVIEW DARI REVIEWERS

Universitas Hasanuddin

**Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>**

# Decision on submission to Journal of Drug Delivery Science and Technology

1 message

**Journal of Drug Delivery Science and Technology** <em@editorialmanager.com> Mon, Aug 1, 2022 at 8:11 AM

Reply-To: Journal of Drug Delivery Science and Technology <support@elsevier.com>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Manuscript Number: JDDST-D-22-01553

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

Dear Dr. Permana,

Thank you for submitting your manuscript to Journal of Drug Delivery Science and Technology.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following major revision. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Sep 29, 2022.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at https://www.editorialmanager.com/jddst/, and navigate to the "Submissions Needing Revision" folder.

Journal of Drug Delivery Science and Technology values your contribution and I look forward to receiving your revised manuscript.

Please include the following two versions of the manuscript when you submit the revision and ensure you remove the original version of the manuscript file. Both the versions can be uploaded using the file type 'Manuscript'.

1. Revised Manuscript with changes marked
2. Revised Manuscript with no changes marked

Kind regards,
Shirui Mao
Associate Editor

Journal of Drug Delivery Science and Technology

Editor and Reviewer comments:

Reviewer #1: An interesting piece of work that adds new knowledge to the field and will, in due course, be widely read and cited. All experiments have been well-planned and interpreted, data analysis is appropriate and conclusions drawn sensible.

The authors should consider the RaScaL study (Suner et al Ophthalmologica. 2014 Nov 26. doi: 10.1159/000367902.) Would the approaches there provide any insights into the current work on ocular drug delivery?

Could the authors possibly provide details of how this work will be translated? How would the system be manufactured at scale? How would patients/clinicians use it? What barriers to success exist? How would it be sterilised without damaging the cargo or crosslinking the polymers? Such assertions are important for readers to understand the potential impact of the work.

The Figure legends are not labelled with means +/- S.D. What is n= ? Such attention to detail is important. Similarly, many words derived from classical languages (e.g. in situ) should be in italics throughout.

Finally, the authors should re-read and improve the scientific English throughout.

Reviewer #2: The drug delivered by the mucoadhesive-thermosensitive gel was an antibiotic, a microbiological assay will be needed to demonstrate the activity of the drug is maintained.

In the ex vivo permeation studies presented in the section 3.7 needs to be analysed in terms of permeation and not using the release kinetic as presented in table 5. To determine the permeation rates and the flux please see the following papers (https://doi.org/10.3390/md15120370; https://doi.org/10.1016/j.ijpharm.2020.119020) to determine those parameters and discuss data considering those parameters.

There are many data repeated in graphics and, in the text, please delete some of the graphics namely the Figure 1, Figure 2c; Figure 4a.

*****

any time. (Use the following URL: https://www.editorialmanager.com/jddst/login.asp?a=r). Please contact the publication office if you have any questions.

# BUKTI SUBMIT HASIL REVIEW

Universitas Hasanuddin

**Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>**

# Confirming submission to Journal of Drug Delivery Science and Technology

1 message

**Journal of Drug Delivery Science and Technology** <em@editorialmanager.com> Mon, Aug 8, 2022 at 10:02 AM

Reply-To: Journal of Drug Delivery Science and Technology <support@elsevier.com>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

*This is an automated message.*

Manuscript Number: JDDST-D-22-01553R1

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

Dear Dr. Permana,

We have received the above referenced manuscript you submitted to Journal of Drug Delivery Science and Technology.

To track the status of your manuscript, please log in as an author at https://www.editorialmanager.com/jddst/, and navigate to the "Revisions Being Processed" folder.

Thank you for submitting your revision to this journal.

Kind regards,
Journal of Drug Delivery Science and Technology

More information and support

You will find information relevant for you as an author on Elsevier's Author Hub: https://www.elsevier.com/authors

FAQ: How can I reset a forgotten password?
https://service.elsevier.com/app/answers/detail/a_id/28452/supporthub/publishing/
For further assistance, please visit our customer service site: https://service.elsevier.com/app/home/supporthub/publishing/
Here you can search for solutions on a range of topics, find answers to frequently asked questions, and learn more about Editorial Manager via interactive tutorials. You can also talk 24/7 to our customer support team by phone and 24/7 by live chat and email

#AU_JDDST#

To ensure this email reaches the intended recipient, please do not delete the above code

---

Manuscript Number: JDDST-D-22-01553

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

**Response to Reviewers**

We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. We have made some changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each of the reviewers' comments in detail below. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript.

**Reviewer #1**: An interesting piece of work that adds new knowledge to the field and will, in due course, be widely read and cited. All experiments have been well-planned and interpreted, data analysis is appropriate and conclusions drawn sensible.

**Response to Reviewer**

We are very thankful to the Reviewer for taking the time to review this manuscript and for the expert review, providing helpful comments. We are glad that the Reviewer thinks that our work is interesting. We have made a number of key changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each one of the reviewers' comments in detail.

The authors should consider the RaScaL study (Suner et al Ophthalmologica. 2014 Nov 26. doi: 10.1159/000367902.) Would the approaches there provide any insights into the current work on ocular drug delivery?

**Response:**

We thank the reviewer for the suggestion. The paper evaluated the efficacy, durability, and safety of a single treatment with intravitreal ranibizumab plus peripheral scatter laser (RaScaL) in patients with diabetic macular edema associated with peripheral retinal nonperfusion on ultrawide-field fluorescein angiography (UWFA). Although the paper did not describe the ocular drug delivery, the paper provides several useful information with regard to the design of clinical study in phase I/II. The clinical study design described could be potentially applied when the system developed in our study reaches the clinical evaluations.

Could the authors possibly provide details of how this work will be translated? How would the system be manufactured at scale? How would patients/clinicians use it? What barriers to success exist? How would it be sterilised without damaging the cargo or crosslinking the polymers? Such assertions are important for readers to understand the potential impact of the work.

**Response:**

We thank the reviewer for the suggestion. Indeed, we agree that the explanations recommended by the Reviewer is crucial. As a result of the reviewer suggestion, we have added the following explanation in the revised manuscript:

The use 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 (Rafael et al., 2019; Raval and Bagada, 2021). 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.

The Figure legends are not labelled with means +/- S.D. What is n= ? Such attention to detail is important. Similarly, many words derived from classical languages (e.g. in situ) should be in italics throughout.

**Response:**

We thank the reviewer for pointing this out. We have added this information in the revised manuscript. Additionally, all words from classical languages are now in italics.

Finally, the authors should re-read and improve the scientific English throughout.

**Response:**

We thank the reviewer for the suggestion. We have re-read the manuscript and have made a great effort to improve the English throughout.

**Reviewer #2**: The drug delivered by the mucoadhesive-thermosensitive gel was an antibiotic, a microbiological assay will be needed to demonstrate the activity of the drug is maintained.

**Response:**

We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. As a result of this comment, we have performed *in vitro* antimicrobial activity against *Pseudomonas aeruginosa*. It was found that the inhibition zones of CFZ solution and CFZ in thermosensitive *in situ* gel were 21.98 ± 1.98 mm and 20.76 ± 1.21 mm. Analyzed statistically, there was no a significant different ($p > 0.05$) between those values, indicating that the formulation did not affect the antimicrobial activity of CFZ against *Pseudomonas aeruginosa*. We have added this in the revised manuscript.

In the ex vivo permeation studies presented in the section 3.7 needs to be analysed in terms of permeation and not using the release kinetic as presented in table 5. To determine the permeation rates and the flux please see the following papers (https://doi.org/10.3390/md15120370; https://doi.org/10.1016/j.ijpharm.2020.119020) to determine those parameters and discuss data considering those parameters.

**Response:**

We thank the reviewer for the suggestions. We have calculated permeation flux and permeation coefficient from our study according to the suggested papers (Silva et al., 2020, 2017) and have included the results in the revised manuscript.

There are many data repeated in graphics and, in the text, please delete some of the graphics namely the Figure 1, Figure 2c; Figure 4a.

**Response:**

We thank the reviewer for the suggestion. We have deleted some graphs as requested.

## References

Rafael, D., Andrade, F., Martinez-Trucharte, F., Basas, J., Seras-Franzoso, J., Palau, M., Gomis, X., Pérez-Burgos, M., Blanco, A., López-Fernández, A., Vélez, R., Abasolo, I., Aguirre, M., Gavaldà, J., Schwartz, S., 2019. Sterilization procedure for temperature-sensitive hydrogels loaded with silver nanoparticles for clinical applications. Nanomaterials 9. https://doi.org/10.3390/nano9030380

Raval, M., Bagada, H., 2021. Formulation and Evaluation of Cyclodextrin-Based Thermosensitive In Situ Gel of Azithromycin for Periodontal Delivery. J. Pharm. Innov. 16, 67–84. https://doi.org/10.1007/s12247-019-09422-3

Silva, B., Marto, J., Braz, B.S., Delgado, E., Almeida, A.J., Gonçalves, L., 2020. New nanoparticles for topical ocular delivery of erythropoietin. Int. J. Pharm. 576, 119020. https://doi.org/10.1016/j.ijpharm.2020.119020

Silva, M.M., Calado, R., Marto, J., Bettencourt, A., Almeida, J., Gonçalves, L.M.D., 2017. Chitosan Nanoparticles as a Mucoadhesive Drug Delivery System for Ocular Administration 1–16. https://doi.org/10.3390/md15120370

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

Muh. Al Fiqri[1], Alhidayah[1], Nirmayanti[1], Ummu Athiyyah[1], Patricia Layadi[1], Tamara Gabriela Angeleve Fadjar[2], Andi Dian Permana[1*]

1. Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia
2. Faculty of Medicine, Hasanuddin University, Makassar, 90245, Indonesia

***Corresponding author:**

Andi Dian Permana

Faculty of Pharmacy, Hasanuddin University, Indonesia

Email: andi.dian.permana@farmasi.unhas.ac.id

## 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.

**Keywords:** Cefazoline, Bacterial keratitis, mucoadhesive-thermosensitive *in situ* gel

## 1. Introduction

Bacterial keratitis is an inflammatory process caused by a bacterial infection. Approximately 64.6% of keratitis cases are caused by bacterial infection. The use of contact lenses has been reported to be the main risk factor of this disease [1]. Previous study has reported that the 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 the 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 anatomic 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 results in very poor bioavailability of the drug [6]. Furthermore, it also causes low drug permeation to 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 vicious 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 vison 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. Also, 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 gifted 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 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* gel formula were carried out.

**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 |

### 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$^{o}$ [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 gram of the metal weight was added every 30 seconds until ocular tissue was separated from other tissue [19,20]

### 2.3.2. pH Evaluation

The pH value was assessed using a digital pH meter.

### 2.3.3. 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$^{o}$C, 37$^{o}$C, and 4$^{o}$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 10 µg/mL. All formulations were then centrifuged for 15 minutes 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 hours and replaced with 1 ml of fresh STF. The release kinetic profile was determined using the following equations [22].

$$Zero\ Order\ Kinetics: Ct = C0 + k0t$$

$$First\ Order\ Kinetics: \ln Ct = \ln C0 + k1t$$

$$Higuchi\ Model: Ct = kHt$$

$$Korsmeyer{-}Peppas\ Model: Ct = kKPtn$$

$$Hixson{-}Crowell\ Model: C_t^{1/3} = C_0^{1/3} k_{HC} t$$

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, 0.75, 1, 2, 3, 4, 5, 6-, 7-, 8-, and 24-hours, 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 drug concentration in the sample was calculated [18]. Afterwards, the fluxes ($J$, µg/cm$^2$/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 at 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 x $10^8$ 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 μg/mL. The media was incubated at 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 preserved in 10% neutral buffered formalin. Then, the tissue was placed in paraffin wax. Sections with a thickness of 4 micrometers 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 ± 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 both polymers 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® 127 127 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 grams each formula was obtained, with the appearance of being clear and liquid at cold temperatures and then turning into a gel when heated (Figure 1A and 1B).

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°C to 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°C to 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].

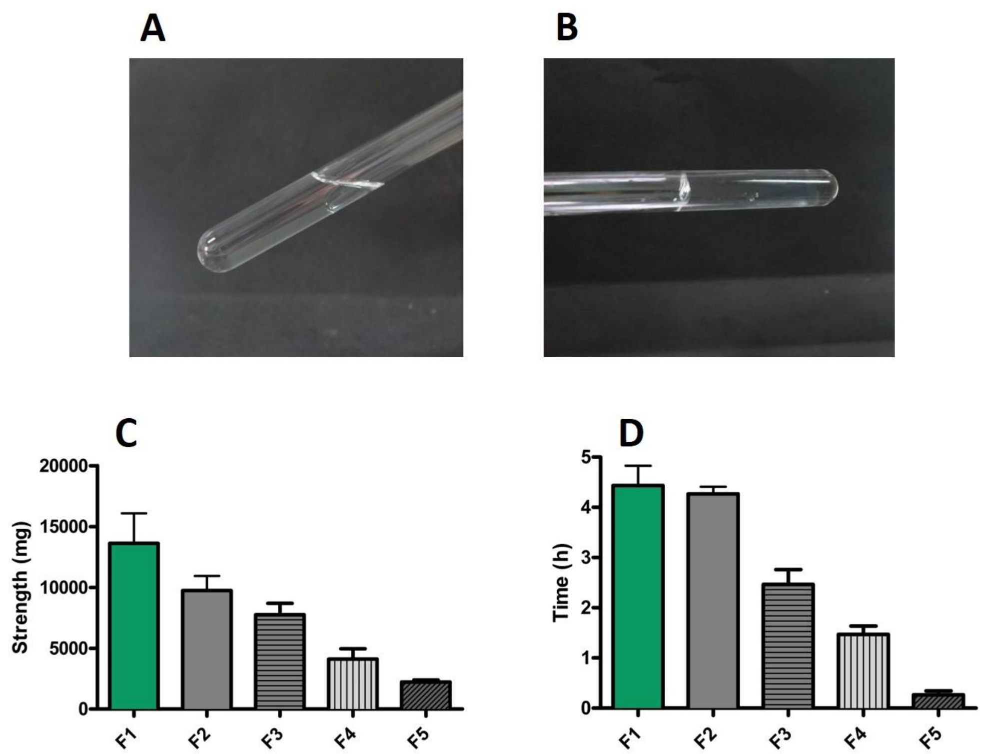


**Figure 1.** CFZ mucoadhesive thermosensitive *in situ* gel formulation in room temperature (A) CFZ mucoadhesive thermosensitive *in situ* gel formulation in body temperature (B). Mucoadhesive strength (C) and mucoadhesive time (D) of five formulations of mucoadhesive thermosensitive *in situ* gel of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

## 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 that discuss this mucoadhesive phenomenon, one of the theories is the adsorption theory. This theory discusses the mechanism of muchoadhesion, including hydrogen bonds, van der Waals, and hydrophobic agents. Although this type is classified as weak bound, but if many bounds 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, muchoadhesive 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 Figure 1C and 1D.

As can be seen in Figure 1C and 1D, 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 to 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 eventually makes many processing methods possible [32]. In this study, the viscosity of the thermosensitive gels was observed at 4 °C (cold temperature), 25 °C (room temperature), and 37 °C (body temperature) (Figure 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 the improve the contact time. With respect to the characteristic of the rheology, commonly, the gel preparation shows pseudoplastic flow. Accordingly, the thermosensitive 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 sharing 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 Figure 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.

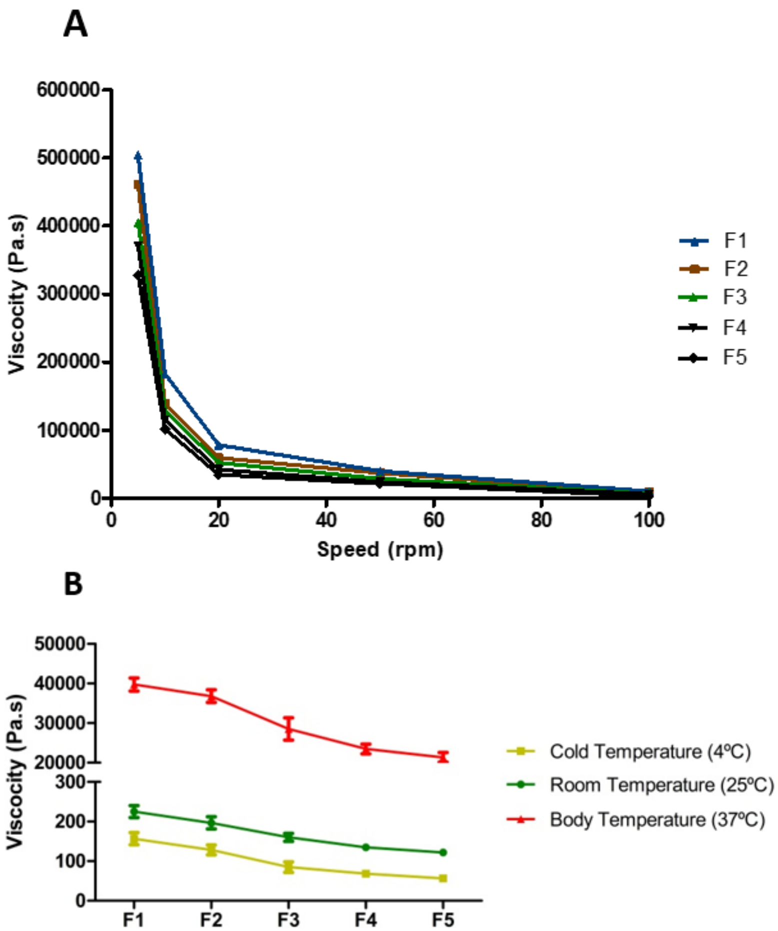


**Figure 2.** Rheology of CFZ mucoadhesive-thermosensitive *in situ* gel formulation with variety of spindle rotation (rpm) (A). Viscosity of CFZ mucoadhesive-thermosensitive *in situ* gel formula in three different temperatures (B) (mean ± SD, $n = 3$).

### 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 form 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 (Figure 4), only after 3 hours, 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 hours 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.

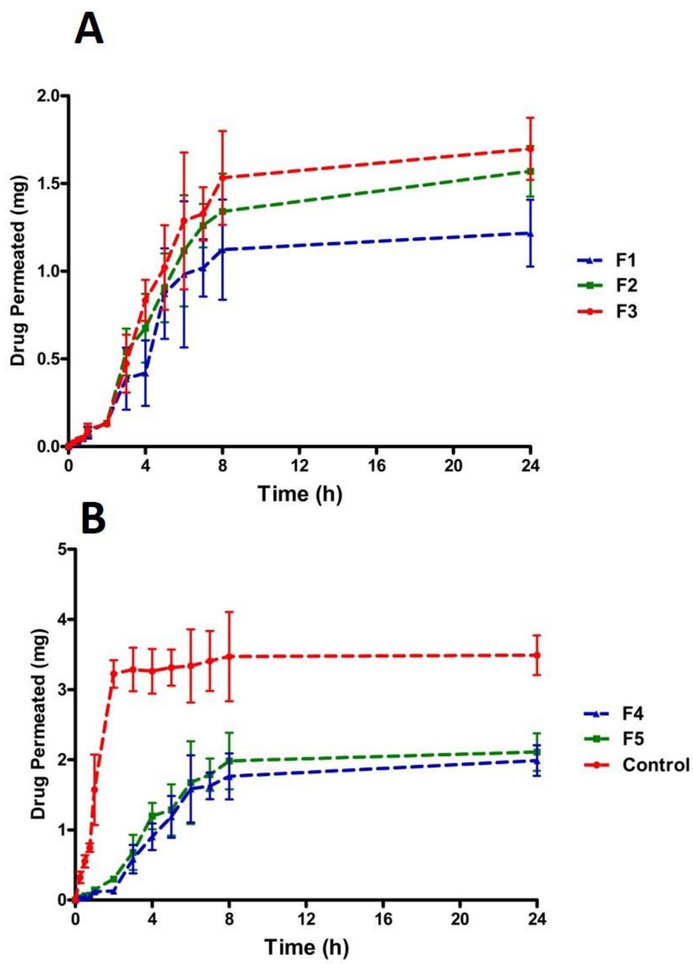


**Figure 3.** *In vitro* permeation study of CFZ thermosensitive muchoadhesive *in situ* gel in dialysis membrane (mean ± SD, *n* = 3).

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.

**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 |

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].

### 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 Figure 4A. From the data, it was shown that after 24 hours, 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 hours 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 (Jss) 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 Jss and *Kp* 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.

**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 |

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 hours of the application of each formulation. The retention results were also compared to control solution and the results are exhibited in Figure 4B.

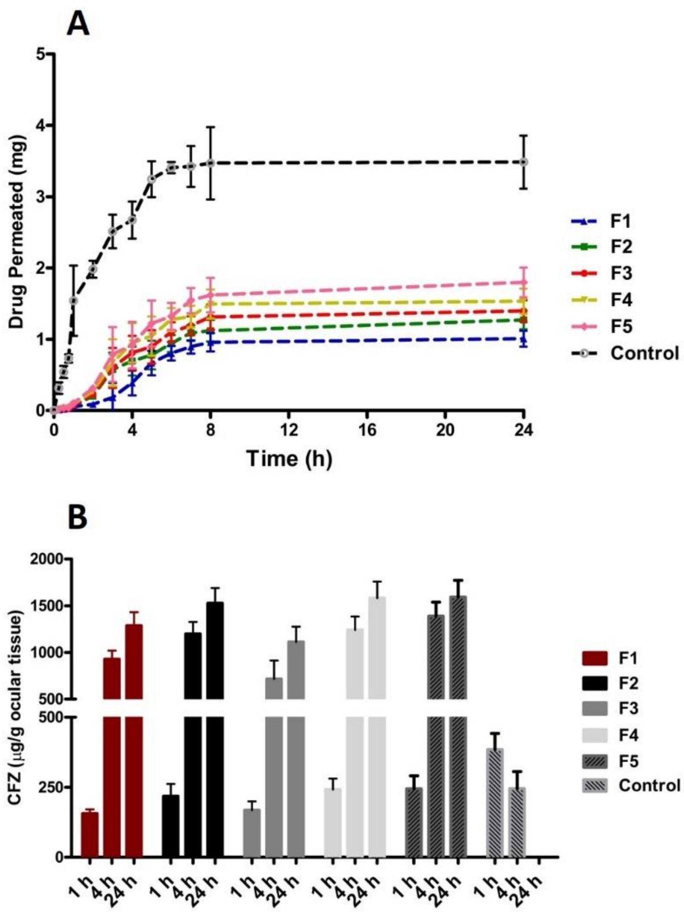


**Figure 4.** *Ex vivo* permeation study of CFZ mucoadhesive thermosensitive *in situ* gel in ocular tissue compared with control eye drop (A). *Ex vivo* retention of mucoadhesive thermosensitive in situ gel and control eye drop of CFZ in ocular tissue (B) (mean ± SD, *n* = 3).

According the bar charts, it was shown that the incorporation CFZ to the thermosensitive hydrogel could increase the location of CFZ in the 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 ± 143.72 µg/g – 1592.19 ± 179.34 µg/g. Interestingly, no CFZ was detected in the case of control solution after 24 h of application. Therefore, the thermosensitive system 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.

**Table 4**. Flux of permeation (Jss) and permeation coefficient (*K*p) of CFZ from mucoadhesive-thermosensitive and control solution (mean ± SD, *n* = 3).

| **Formulation** | **Jss (μg/(cm$^2$h))** | **Kp (cm/h)** |
|---|---|---|
| F1 | 127.23 ± 11.98 | 0.054 ± 0.001 |
| F2 | 133.93 ± 10.39 | 0.065 ± 0.001 |
| F3 | 149.11 ± 12.13 | 0.078 ± 0.002 |
| F4 | 154.17 ± 13.09 | 0.087 ± 0.002 |
| F5 | 176.27 ± 16.12 | 0.093 ± 0.001 |
| Control solution | 687.23 ± 41.23 | 0.233 ± 0.01 |

### 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 Figure 5A and 5B, showing that the hemolysis percentages were below 1.5% in all concentrations tested. Accordingly, the formulations developed in this study were considerably safe.

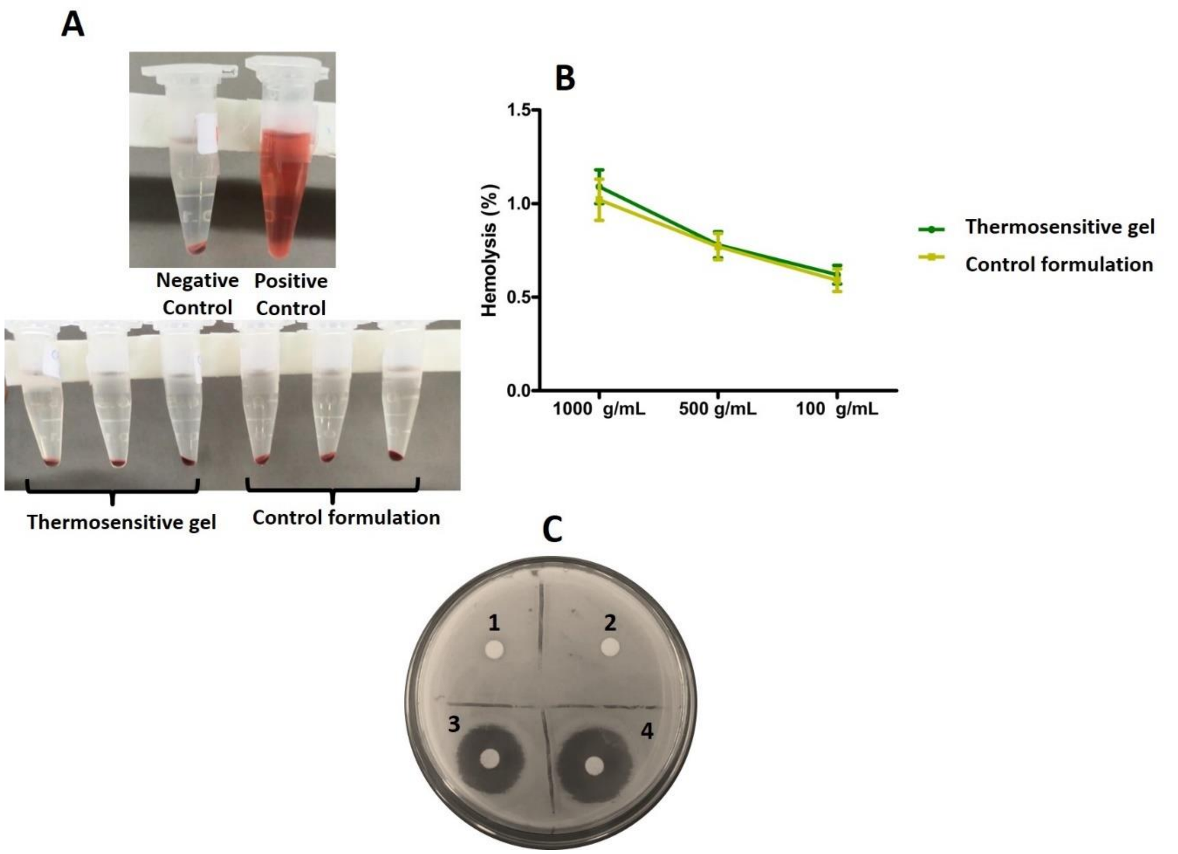


**Figure 5.** Representative images of *in vitro* hemolysis study in red blood cells (A). Hemolysis percentages of thermosensitive and control formulations (B) (mean ± SD, $n = 3$). Inhibition zones of blank disc (1), water (2), CFZ solution (3) and CFZ thermosensitive formulation against *Pseudomonas aeruginosa* (C).

## 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*. Figure 5C depicts the representative image of this study. It was found that the inhibition zones of CFZ solution and CFZ in thermosensitive *in situ* gel were 21.98 ± 1.98 mm and 20.76 ± 1.21 mm. Analyzed statistically, there was no a significant different ($p > 0.05$) between those values, indicating that the formulation did not affect the antimicrobial activity of CFZ against *Pseudomonas aeruginosa*.

### 3.10. *In vivo* irritation evaluation

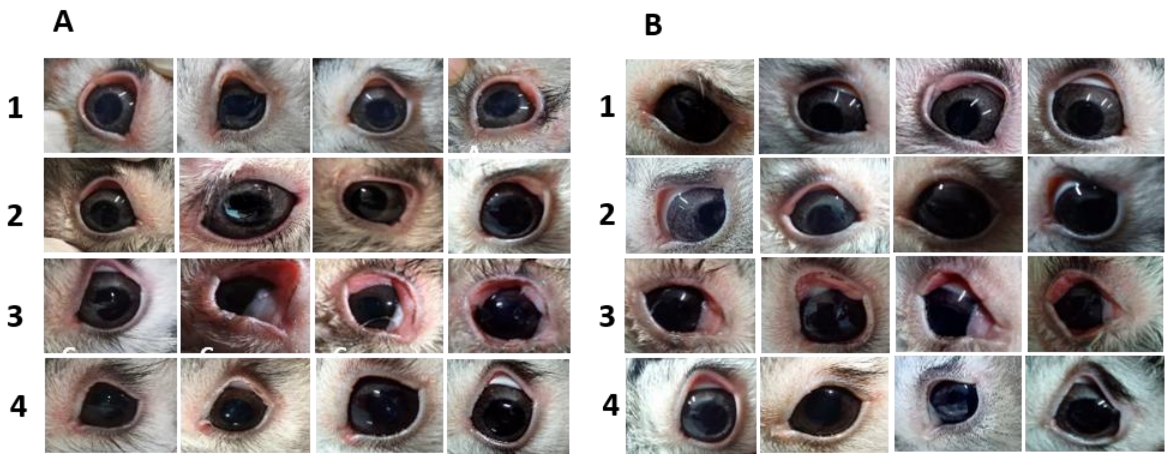


**Figure 6.** Rabbit's eye on day one treatment (A) and last day treatment (B), (1 = with CFZ), (B = With NaCl 0,9%), (C = with Sodium lauryl sulfate (SLS)), (D = without treatment).

We further assess the irritation of our approach *in vivo* in rabbits. Figure 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 Figure 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.

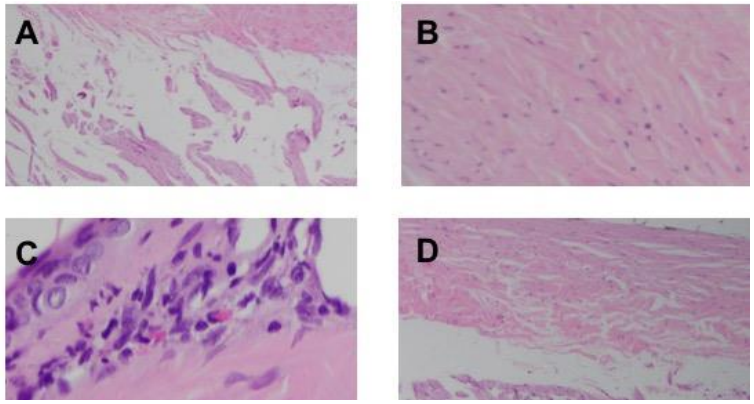


**Figure 7.** Histopathological result of (A) NaCl 0,9% solution; (B) CFZ mucoadhesive-thermosensitive in situ gel formula; (C) sodium lauryl sulfate (SLS) solution; and (D) without treatment

Overall outcomes have proven that the incorporation of CFZ into themosensitive-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 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 of the preparation with the eye up to 4 hours. 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 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 Athiyyah:** 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.

**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 Pluronics®.

## 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. Yildirim-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. Ther. 36 (2020) 342–354.

[7] M.F. Rozi, A.S. Mohmad Sabere, Review on Conventional and Novel Topical Ocular Drug Delivery System, J. Pharm. 1 (2021) 19–26.

[8] S.A. Young, H. Riahinezhad, 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-Pîrvu, M.V. Ghica, D. Lupuleasa, 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. Vigani, 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-Jouzdani, J.D. Wolf, F. Atyabi, A. Bernkop-Schnürch, In situ gelling and mucoadhesive polymers: why do they need each other?, Expert 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. Athiyyah, 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) 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/acsami.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 antifilariasis 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.

# BUKTI ACCEPTED

Universitas Hasanuddin

**Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>**

# Decision on submission to Journal of Drug Delivery Science and Technology

1 message

**Journal of Drug Delivery Science and Technology** <em@editorialmanager.com> Mon, Aug 29, 2022 at 3:45 PM

Reply-To: Journal of Drug Delivery Science and Technology <support@elsevier.com>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Manuscript Number: JDDST-D-22-01553R1

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

Dear Dr. Permana,

Thank you for submitting your manuscript to Journal of Drug Delivery Science and Technology.

I am pleased to inform you that your manuscript has been accepted for publication.

My comments, and any reviewer comments, are below.
Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Journal of Drug Delivery Science and Technology and hope you will consider us again for future submissions.

Kind regards,
Shirui Mao
Associate Editor

Journal of Drug Delivery Science and Technology

Editor and Reviewer comments:

More information and support

FAQ: When and how will I receive the proofs of my article?
https://service.elsevier.com/app/answers/detail/a_id/6007/p/10592/supporthub/publishing/related/

You will find information relevant for you as an author on Elsevier's Author Hub: https://www.elsevier.com/authors

FAQ: How can I reset a forgotten password?
https://service.elsevier.com/app/answers/detail/a_id/28452/supporthub/publishing/
For further assistance, please visit our customer service site: https://service.elsevier.com/app/home/supporthub/publishing/
Here you can search for solutions on a range of topics, find answers to frequently asked questions, and learn more about Editorial Manager via interactive tutorials. You can also talk 24/7 to our customer support team by phone and 24/7 by live chat and email

#AU_JDDST#

To ensure this email reaches the intended recipient, please do not delete the above code

---

In compliance with data protection regulations, you may request that we remove your personal registration details at any time.  (Use the following URL: https://www.editorialmanager.com/jddst/login.asp?a=r). Please contact the publication office if you have any questions.