# BUKTI SUBMIT MANUSKRIP

Universitas Hasanuddin

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

# A manuscript number has been assigned: COLSUB-D-22-01410

1 message

**Colloids and Surfaces B** <em@editorialmanager.com> Sat, Jul 9, 2022 at 6:40 PM
Reply-To: Colloids and Surfaces B <support@elsevier.com>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Ms. Ref. No.: COLSUB-D-22-01410
Title: Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®
Colloids and Surfaces B: Biointerfaces

Dear Dr. Permana,

Your submission, referenced above, has been assigned the manuscript number COLSUB-D-22-01410 and has been assigned to an Editor who will handle peer review.

Please note that in most cases at least two reviews may be required before a decision on a manuscript is made. You will be notified by e-mail each time a reviewer agrees to review your manuscript.

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Kind regards,

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# BUKTI REVIEW DARI REVIEWERS

Universitas Hasanuddin

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

# Your Submission COLSUB-D-22-01410 - Revision due Aug 23, 2022

1 message

**Deborah Leckband** <em@editorialmanager.com> Wed, Aug 3, 2022 at 7:55 AM
Reply-To: Deborah Leckband <leckband@illinois.edu>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Ms. Ref. No.: COLSUB-D-22-01410
Title: Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®
Colloids and Surfaces B: Biointerfaces

Dear Dr. Andi Dian Permana,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript.

To submit a revision, please go to https://www.editorialmanager.com/colsub/ and login as an Author.
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When submitting your revised paper, we ask that you include the following items:

Manuscript and Figure Source Files (mandatory)

We cannot accommodate PDF manuscript files for production purposes. We also ask that when submitting your revision you follow the journal formatting guidelines. Figures and tables may be embedded within the source file for the submission as long as they are of sufficient visual quality. For any figure that cannot be embedded within the source file (such as *.PSD Photoshop files), the original figure needs to be uploaded separately. Refer to the Guide for Authors for additional information.

Highlights (mandatory)

Highlights consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See the following website for more information http://www.elsevier.com/highlights

Graphical Abstract (mandatory)

Graphical Abstracts should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Refer to the following website for more information: http://www.elsevier.com/graphicalabstracts

Include interactive data visualizations in your publication and let your readers interact and engage more closely with your research. Follow the instructions here: https://www.elsevier.com/authors/author-services/data-visualization to find out about available data visualization options and how to include them with your article.

Yours sincerely,

Deborah Leckband, PhD
Editor
Colloids and Surfaces B: Biointerfaces

Reviewers' comments:

Reviewer #1: The paper "Enhanced and sustained transdermal delivery of primaquine from 1 polymeric thermoresponsive 2 hydrogels in combination with Dermarollers®" focuses on the use of hydrogels as effective system for the topical delivery of primaquine. The use of Dermarollers seemed to be a further strategy capable of promoting the effectiveness of the drug against malaria infections. The paper is interesting, however some modification and especially different improvement, are needed before to be accepted for publication in this Journal.

The abstract should report more information, especially regarding the physico-chemical properties of the hydrogel. More details about the difficulties to use primaquine in therapy should be reported! Which modification of the molecular structure of primaquine have been detected after oral administration? Which kind of side effects may be associate with the use of primaquine?
The introduction should report more information about the polymers selected.
"Determination of gelation temperature and bioadhesive properties", this section should contain more details none of the described technique is understandable especially for unexpert people, please check and improve.
The duration of the release studies must be reported along with more details about the technique chosen.
For in vitro permeation studies, the receptor fluid has been also assayed? Withdrawals have been performed during the experiment? Please had more details also in this paragraph.
Results and discussion section reports only the results without any discussion to explain the behavior of these systems. This section needs to be significantly improved and some comparison with previous paper reporting similar studies should be reported.
I also suggest a careful check of the English language, a mother tongue is neded to improve the quality of the paper also from a language point of view.

Reviewer #2: This is a nice piece of work that will add to knowledge in the field. The study has clearly been well-planned, carefully executed and data meticulously analysed. Statistical treatment of data is appropriate and the conclusions drawn are sensible.

This work will add to the ever-growing body of evidence on the effectiveness of microneedle systems for drug delivery. The paper is likely to be widely read and, in due course, cited.

I recall a study from Wi & Rascal on transdermal delivery of primaquine from several years ago. The approach described in the present work goes, in my opinion, substantially beyond that, showing in situ depot formation, with several days' delivery now possible, instead of 24 hours' permeation. This shows some inventiveness.

The authors should consider translation of this technology. Will regulatory bodies demand sterility of the topical gel, given that it is intended for application to microneedle-treated skin? What manufacturing and distribution challenges will this present? What about storage stability? How would this Dermaroller + topical gel process be reproducibly performed by patients or their carer? How would the correct dose be delivered every time? What about disposal of the Dermaroller to avoid infection on subsequent applications? Maybe it would be sterilised between uses? How would this be done safely and securely in resource-poor settings?

Microneedle pre-treatment plus topical gel application deposit polymers in skin. How quickly would it biodegrade/be cleared? Would it accumulate in skin or the draining lymph node local to the site of application? How would it be excreted? These are important translational considerations, as is scaled-up manufacture. Would the described production method really be suitable for manufacture of the numbers of gels required for a commercialised product?

In the bar graphs, the authors rightly show positive error bars for clarity. However, Figure legends say "+/- S.D." This should be corrected. In Figure 2, panel D needs to be labelled in the legend. The (D) is missing at the end of the text, which could cause some confusion. Finally, the authors should re-read and improve the scientific English throughout.

# BUKTI SUBMIT HASIL REVIEW

Universitas Hasanuddin

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

# Submission Confirmation for: COLSUB-D-22-01410R1

1 message

**Colloids and Surfaces B** <em@editorialmanager.com> Tue, Aug 9, 2022 at 12:15 PM
Reply-To: Colloids and Surfaces B <support@elsevier.com>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Ms. Ref. No.: COLSUB-D-22-01410R1
Title: Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®
Colloids and Surfaces B: Biointerfaces

Dear Dr. Andi Dian Permana,

Your revised manuscript has been received for reconsideration for publication in Colloids and Surfaces B: Biointerfaces under the Short Communication category.

You may check the status of your manuscript by logging onto the Editorial Manager of the journal as an Author. at

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For further assistance, please visit our customer support site at http://help.elsevier.com/app/answers/list/p/7923 Here you can search for solutions on a range of topics, find answers to frequently asked questions and learn more about EM via interactive tutorials. You will also find our 24/7 support contact details should you need any further assistance from one of our customer support representatives.

Yours sincerely,

Editorial Manager
Colloids and Surfaces B: Biointerfaces

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Colloids and Surfaces B: Biointerfaces

# Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®

--Manuscript Draft--

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|---|---|
| **Manuscript Number:** | COLSUB-D-22-01410R1 |
| **Article Type:** | Short Communication |
| **Keywords:** | Primaquine; thermoresponsive; Transdermal; Dermarollers® |
| **Corresponding Author:** | Andi Dian Permana, Ph.D<br><br>INDONESIA |
| **First Author:** | Andi Dian Permana, Ph.D |
| **Order of Authors:** | Andi Dian Permana, Ph.D |
| | Diany Elim |
| | Putri Wulandari Resky Ananda |
| | Hilman Syamami Zaman |
| | Wahdaniyah Muslimin |
| | Muhamad Gilang Ramadhan Tunggeng |
| **Manuscript Region of Origin:** | INDONESIA |
| **Abstract:** | Primaquine (PMQ) is an effective antimalaria drug with several limitations. We report the combinatorial approach of thermoresponsive hydrogels and Dermarollers ® for transdermal delivery of PMQ to overcome these limitations. The hydrogels were prepared using Pluronic F127 (PF127) and F68 (PF68). Specifically, HPMC was added into the formulation to improve the bioadhesive properties. Numerous formulations were prepared, showing that formulation comprising 15% PF127, 3% PF68 and 0.4% HPMC with 1% PMQ was selected as the optimum formulation. The formulation showed the gelation temperature around 35°C with bioadhesive strength of 26.43 ± 2.31 dyne.cm 2 . Importantly, the pH of the formulation was suitable for skin application with the percentage of PMQ recovery of 99.57 ± 3.23%. Moreover, the hydrogels exhibited free-flow liquid at storage and room temperature and high viscosities in the skin temperature. In vitro release experiments showed that the release of PMQ was sustained for 24 h. Evaluated in extensive ex vivo studies, the treatment with Dermarollers ® improved the skin permeation and retention of PMQ for 3 days. In combination with Dermarollers ® , the ex vivo permeation of PMQ was sustained and the localization of PMQ in the skin was improved over 72 h. |
| **Suggested Reviewers:** | Ryan Donnelly<br>r.donnelly@qub.ac.uk |
| | Aaron Courtenay<br>a.courtenay@ulster.ac.uk |
| | Eneko Larraneta<br>e.larraneta@qub.ac.uk |
| | Juan Dominguez Robles<br>J.DominguezRobles@qub.ac.uk |
| | Jainer Pasca Siampa<br>jainerpsiampa@unsrat.ac.id |
| | Ahmed Faheem<br>ahmed.faheem@sunderland.ac.uk |
| **Response to Reviewers:** | Ms. Ref. No.: COLSUB-D-22-01410<br>Title: Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®<br>Colloids and Surfaces B: Biointerfaces |

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:
The paper "Enhanced and sustained transdermal delivery of primaquine from 1 polymeric thermoresponsive 2 hydrogels in combination with Dermarollers®" focuses on the use of hydrogels as effective system for the topical delivery of primaquine. The use of Dermarollers seemed to be a further strategy capable of promoting the effectiveness of the drug against malaria infections. The paper is interesting, however some modification and especially different improvement, are needed before to be accepted for publication in this Journal.
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 abstract should report more information, especially regarding the physico-chemical properties of the hydrogel.
Response:
We thank the Reviewer for the suggestions. We have included more information regarding the physico-chemical characterizations of the hydrogel in the abstract in the revised manuscript.

More details about the difficulties to use primaquine in therapy should be reported! Which modification of the molecular structure of primaquine have been detected after oral administration? Which kind of side effects may be associate with the use of primaquine?
Response:
We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:
After oral administration, PMQ undergoes first-pass metabolism in the liver, forming carboxyprimaquine (cPQ) through oxidative deamination. This metabolite has been found to show less antimalarial effect and high haemotoxic effect. Additionally, other side effects have been also found following oral administration of PMQ, including abdominal pain, bitter taste, nausea and long therapy duration [1,2].

The introduction should report more information about the polymers selected.
Response:
We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:
Several polymers have been explored to prepare this approach. Amongst numerous polymers, in this study, Pluronic® was used as the thermoresponsive agent [3]. The properties of this polymer are sensitive to temperatures, making it suitable for the development of thermosensitive hydrogels with various applications. Importantly, Pluronic® is compatible with several type of cells and biological fluids, as well as non-irritant [4–6]. To improve its effectiveness, bioadhesive agents can be included into thermoresponsive formulations. Hydroxy propyl methyl cellulose (HPMC) has been recognized to show this property [7]. It has been reported that, combined with microneedles, this system has successfully improved the penetrability of fluorescein sodium [8].

"Determination of gelation temperature and bioadhesive properties", this section should contain more details none of the described technique is understandable especially for unexpert people, please check and improve.
Response:
We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:

The tube containing the formulation was placed at 20°C. The temperature was steadily increased until 55°C. The gelation temperature was denoted when the solution turned into a gel after turning the tube over to 90° for 30 s. To determine the bioadhesion strength in skin tissue, a modified physical balance was applied [4]. In this study, an abdomen part of rats' skin was used. The skin was shaved and washed using distilled water. Prior to the experiment, the formulations were placed at 37°C for 10 minutes and then applied to the skin attached to the balance in one side. Afterward, weights were placed in the other side of the balance until the formulations were detached from the skin. Finally, the bioadhesive strength was determined using the following calculation:
Bioadhesive strength = (weight (g)x 100)/(surface area ( 〖cm〗 ^(2)) ) x 9.81

The duration of the release studies must be reported along with more details about the technique chosen.
Response:
We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:
The in vitro release of PMQ from thermoresponsive hydrogel was carried out using membraneless dissolution method using phosphate-buffered saline (PBS) as release media. Briefly, 10 g of hydrogel was placed into orbital shaker at 37 ± 0.5 °C. After the formulation became gel, PBS (5 mL) was added into the gels. At predetermined time (0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 12 h and 24 h), 1 mL of medium was taken and replaced with fresh medium.

For in vitro permeation studies, the receptor fluid has been also assayed? Withdrawals have been performed during the experiment? Please had more details also in this paragraph.
Response:
We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:
The ex vivo permeation of PMQ from thermoresporesponsive hydrogels was performed through rats' skin using vertical Franz diffusion cell with PBS as media. The skin was placed between donor and receiver compartment of Franz diffusion cell. The diffusion cell was stirred at 100 rpm and the temperature was maintained at 37 ± 0.5 °C. The formulation (equal 10 mg of PMQ) was placed into donor compartment. With total time of 72 h, at predetermined time, 0.5 mL of permeation media was withdrawn and replaced with new media. In this study, the effect of skin pretreatment with various length of Dermarollers®, namely 0.5 mm, 1 mm and 1.5 mm, was evaluated. Moreover, the concentration of PMQ retained in the skin was determined by extracting PMQ from the skin using methanol and analyzed using spectrophotometer UV-Vis at 265 nm.
Results and discussion section reports only the results without any discussion to explain the behavior of these systems. This section needs to be significantly improved and some comparison with previous paper reporting similar studies should be reported.
Response:
We thank the Reviewer for the suggestions. We have improved the results and discussion section by explaining the behaviour of the system in all characterizations. Furthermore, we have compared our results with previous papers. We believe that the manuscript is now substantially improved

I also suggest a careful check of the English language, a mother tongue is neded to improve the quality of the paper also from a language point of view.
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: This is a nice piece of work that will add to knowledge in the field. The study has clearly been well-planned, carefully executed and data meticulously analysed. Statistical treatment of data is appropriate and the conclusions drawn are sensible.

| | |
|---|---|
| | This work will add to the ever-growing body of evidence on the effectiveness of microneedle systems for drug delivery. The paper is likely to be widely read and, in due course, cited.<br>Response to Reviewer<br>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 clearly been well-planned, carefully executed. 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.<br><br>I recall a study from Wi & Rascal on transdermal delivery of primaquine from several years ago. The approach described in the present work goes, in my opinion, substantially beyond that, showing in situ depot formation, with several days' delivery now possible, instead of 24 hours' permeation. This shows some inventiveness.<br>Response:<br>We thank the Reviewer for the comment. Indeed, PMQ was developed in nanoemulsion delivery system [1]. It was reported that the nanoemulsion could improve and control the transdermal delivery for 24 h. In our study, the sustained release profile was maintained over 72 h. This was due to the formation of in situ depot from thermoresponsive system which allowed the improvement of control release pattern, indicating the inventiveness of our approach. We have included this in the revised manuscript.<br>The authors should consider translation of this technology. Will regulatory bodies demand sterility of the topical gel, given that it is intended for application to microneedle-treated skin? What manufacturing and distribution challenges will this present? What about storage stability? How would this Dermaroller + topical gel process be reproducibly performed by patients or their carer? How would the correct dose be delivered every time? What about disposal of the Dermaroller to avoid infection on subsequent applications? Maybe it would be sterilised between uses? How would this be done safely and securely in resource-poor settings?<br>Response:<br>We thank the Reviewer for the comment and very useful insight. As a result, we have added several information into the revised manuscript. Before this approach can applied in the clinical treatment, several considerations are required. The sterility of this system should be considered. With regard to this, previous study has shown that the penetration of microorganism across the epidermis following the application of microneedles was found to be negligible [9]. Therefore, we could assume that the pores created by the use of microneedle should not cause the risk of infection. However, further studies are required to ensure this in the clinical applications. Moreover, the storage stability of the thermoresponsive gel should be investigated. It is also critical to ensure the reproducibility of this combination approach performed by patients. To achieve this, the pressure applied should be similar. Automated dermaroller has been developed to ensure the reproducibility of pressure applied [10], which could be potentially combined with the thermoresponsive developed in this study. Following this promising results, further in vivo studies should be carried out to investigate the efficacy, the pharmacokinetic profile and the dose determination of this approach.<br><br>Microneedle pre-treatment plus topical gel application deposit polymers in skin. How quickly would it biodegrade/be cleared? Would it accumulate in skin or the draining lymph node local to the site of application? How would it be excreted? These are important translational considerations, as is scaled-up manufacture. Would the described production method really be suitable for manufacture of the numbers of gels required for a commercialised product?<br>Response:<br>We thank the Reviewer for the comment and very useful insight. As a result, we have added several information into the revised manuscript. Moving forward, the impact of the deposition of the polymers should be evaluated. The use of Pluronics-based in the skin administration following microneedle applications is still limited. Therefore, the attention should not only be given to the pharmacokinetic profiles of the drugs, but also to the polymers used, particularly in the clearance profile. Finally, the scale-up manufacturing process should be considered in order to ensure that the method preparation can be applied in the industrial scale. |

In the bar graphs, the authors rightly show positive error bars for clarity. However, Figure legends say "+/- S.D." This should be corrected. In Figure 2, panel D needs to be labelled in the legend. The (D) is missing at the end of the text, which could cause some confusion.
Response:
We thank the Reviewer for pointing this out. To avoid some confusion, we have corrected this part in the revised manuscript.
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.

References:

[1]P. Sharma, M. Tailang, Design, optimization, and evaluation of hydrogel of primaquine loaded nanoemulsion for malaria therapy, Futur. J. Pharm. Sci. 6 (2020). https://doi.org/10.1186/s43094-020-00035-z.
[2]G. Mihaly, S. Ward, G. Edwards, D. Nicholl, M. Orme, A. Breckenridge, Pharmacokinetics of primaquine in man. I. Studies of the absolute bioavailability and effects of dose size., Br. J. Clin. Pharmacol. 19 (1985) 745–750. https://doi.org/10.1111/j.1365-2125.1985.tb02709.x.
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[4]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.
[5]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.
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[7]L. Rahman, R.S. Lembang, S. Lallo, S.R. Handayani, Usmanengsi, A.D. Permana, Bioadhesive dermal patch as promising approach for improved antibacterial activity of bioactive compound of Zingiber cassumunar Roxb in ex vivo Staphylococcus aureus skin infection model, J. Drug Deliv. Sci. Technol. 63 (2021) 102522. https://doi.org/10.1016/j.jddst.2021.102522.
[8]S. Khan, M.U. Minhas, I.A. Tekko, R.F. Donnelly, R.R.S. Thakur, Evaluation of microneedles-assisted in situ depot forming poloxamer gels for sustained transdermal drug delivery, Drug Deliv. Transl. Res. 9 (2019) 764–782. https://doi.org/10.1007/s13346-019-00617-2.
[9]R.F. Donnelly, T.R.R. Singh, A.Z. Alkilani, M.T.C. McCrudden, S. O'Neill, C. O'Mahony, K. Armstrong, N. McLoone, P. Kole, A.D. Woolfson, Hydrogel-forming microneedle arrays exhibit antimicrobial properties: Potential for enhanced patient safety, Int. J. Pharm. 451 (2013) 76–91. https://doi.org/10.1016/j.ijpharm.2013.04.045.
[10]G. Albon, Safety and Effectiveness of an Automated Microneedling Device in Improving the Signs of Aging Skin, 11 (2018) 29–34.

**Declaration of interests**

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ms. Ref. No.: COLSUB-D-22-01410

Title: Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®

Colloids and Surfaces B: Biointerfaces

**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.

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The introduction should report more information about the polymers selected.

**Response:**

We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:

Several polymers have been explored to prepare this approach. Amongst numerous polymers, in this study, Pluronic® was used as the thermoresponsive agent [3]. The properties of this polymer are sensitive to temperatures, making it suitable for the development of thermosensitive hydrogels with various applications. Importantly, Pluronic® is compatible with several type of cells and biological fluids, as well as non-irritant [4–6]. To improve its effectiveness, bioadhesive agents can be included into thermoresponsive formulations. Hydroxy propyl methyl cellulose (HPMC) has been recognized to show this property [7]. It has been reported that, combined with microneedles, this system has successfully improved the penetrability of fluorescein sodium [8].

"Determination of gelation temperature and bioadhesive properties", this section should contain more details none of the described technique is understandable especially for unexpert people, please check and improve.

**Response:**

We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:

The tube containing the formulation was placed at 20°C. The temperature was steadily increased until 55°C. The gelation temperature was denoted when the solution turned into a gel after turning the tube over to 90° for 30 s. To determine the bioadhesion strength in skin tissue, a modified physical balance was applied [4]. In this study, an abdomen part of rats' skin was used. The skin was shaved and washed using distilled water. Prior to the experiment, the formulations were placed at 37°C for 10 minutes and then applied to the skin attached to the balance in one side. Afterward, weights were placed in the other side of the balance until the formulations were detached from the skin. Finally, the bioadhesive strength was determined using the following calculation:

$$\text{Bioadhesive strength} = \frac{weight\ (g) x\ 100}{surface\ area\ (cm^{2})} \text{ x } 9.81$$

The duration of the release studies must be reported along with more details about the technique chosen.

**Response:**

We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:

The *in vitro* release of PMQ from thermoresponsive hydrogel was carried out using membraneless dissolution method using phosphate-buffered saline (PBS) as release media. Briefly, 10 g of hydrogel was placed into orbital shaker at 37 ± 0.5 °C. After the formulation became gel, PBS (5 mL) was added into the gels. At predetermined time (0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 12 h and 24 h), 1 mL of medium was taken and replaced with fresh medium.

For in vitro permeation studies, the receptor fluid has been also assayed? Withdrawals have been performed during the experiment? Please had more details also in this paragraph.

**Response:**

We thank the Reviewer for the suggestions. We have included these in the revised manuscript, as follows:

The *ex vivo* permeation of PMQ from thermoresporesponsive hydrogels was performed through rats' skin using vertical Franz diffusion cell with PBS as media. The skin was placed between donor and receiver compartment of Franz diffusion cell. The diffusion cell was stirred at 100 rpm and the temperature was maintained at 37 ± 0.5 °C. The formulation (equal 10 mg of PMQ) was placed into donor compartment. With total time of 72 h, at predetermined time, 0.5 mL of permeation media was withdrawn and replaced with new media. In this study, the effect of skin pretreatment with various length of Dermarollers®, namely 0.5 mm, 1 mm and 1.5 mm, was evaluated. Moreover, the concentration of PMQ retained in the skin was determined by extracting PMQ from the skin using methanol and analyzed using spectrophotometer UV-Vis at 265 nm.

Results and discussion section reports only the results without any discussion to explain the behavior of these systems. This section needs to be significantly improved and some comparison with previous paper reporting similar studies should be reported.

**Response:**

We thank the Reviewer for the suggestions. We have improved the results and discussion section by explaining the behaviour of the system in all characterizations. Furthermore, we have compared our results with previous papers. We believe that the manuscript is now substantially improved

I also suggest a careful check of the English language, a mother tongue is neded to improve the quality of the paper also from a language point of view.

**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**: This is a nice piece of work that will add to knowledge in the field. The study has clearly been well-planned, carefully executed and data meticulously analysed. Statistical treatment of data is appropriate and the conclusions drawn are sensible.

This work will add to the ever-growing body of evidence on the effectiveness of microneedle systems for drug delivery. The paper is likely to be widely read and, in due course, cited.

**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 clearly been well-planned, carefully executed. 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.

I recall a study from Wi & Rascal on transdermal delivery of primaquine from several years ago. The approach described in the present work goes, in my opinion, substantially beyond that, showing in situ depot formation, with several days' delivery now possible, instead of 24 hours' permeation. This shows some inventiveness.

**Response:**

We thank the Reviewer for the comment. Indeed, PMQ was developed in nanoemulsion delivery system [1]. It was reported that the nanoemulsion could improve and control the transdermal delivery for 24 h. In our study, the sustained release profile was maintained over 72 h. This was due to the formation of *in situ* depot from thermoresponsive system which allowed the improvement of control release pattern, indicating the inventiveness of our approach. We have included this in the revised manuscript.

The authors should consider translation of this technology. Will regulatory bodies demand sterility of the topical gel, given that it is intended for application to microneedle-treated skin? What manufacturing and distribution challenges will this present? What about storage stability?

How would this Dermaroller + topical gel process be reproducibly performed by patients or their carer? How would the correct dose be delivered every time? What about disposal of the Dermaroller to avoid infection on subsequent applications? Maybe it would be sterilised between uses? How would this be done safely and securely in resource-poor settings?

**Response:**

We thank the Reviewer for the comment and very useful insight. As a result, we have added several information into the revised manuscript. Before this approach can applied in the clinical treatment, several considerations are required. The sterility of this system should be considered. With regard to this, previous study has shown that the penetration of microorganism across the epidermis following the application of microneedles was found to be negligible [9]. Therefore, we could assume that the pores created by the use of microneedle should not cause the risk of infection. However, further studies are required to ensure this in the clinical applications. Moreover, the storage stability of the thermoresponsive gel should be investigated. It is also critical to ensure the reproducibility of this combination approach performed by patients. To achieve this, the pressure applied should be similar. Automated dermaroller has been developed to ensure the reproducibility of pressure applied [10], which could be potentially combined with the thermoresponsive developed in this study. Following this promising results, further *in vivo* studies should be carried out to investigate the efficacy, the pharmacokinetic profile and the dose determination of this approach.

Microneedle pre-treatment plus topical gel application deposit polymers in skin. How quickly would it biodegrade/be cleared? Would it accumulate in skin or the draining lymph node local to the site of application? How would it be excreted? These are important translational considerations, as is scaled-up manufacture. Would the described production method really be suitable for manufacture of the numbers of gels required for a commercialised product?

**Response:**

We thank the Reviewer for the comment and very useful insight. As a result, we have added several information into the revised manuscript. Moving forward, the impact of the deposition of the polymers should be evaluated. The use of Pluronics-based in the skin administration following microneedle applications is still limited. Therefore, the attention should not only be given to the pharmacokinetic profiles of the drugs, but also to the polymers used, particularly in the clearance profile. Finally, the scale-up manufacturing process should be considered in order to ensure that the method preparation can be applied in the industrial scale.

In the bar graphs, the authors rightly show positive error bars for clarity. However, Figure legends say "+/- S.D." This should be corrected. In Figure 2, panel D needs to be labelled in the legend. The (D) is missing at the end of the text, which could cause some confusion.

**Response:**

We thank the Reviewer for pointing this out. To avoid some confusion, we have corrected this part in the revised manuscript.

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.

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# Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®

Andi Dian Permana*, Diany Elim, Putri Wulandari Resky Ananda, Hilman Syamami Zaman, Wahdaniyah Muslimin, Muhamad Gilang Ramadhan Tunggeng

**Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia**

***Corresponding author:**
Andi Dian Permana
Faculty of Pharmacy, Hasanuddin University, Indonesia
Email: andi.dian.permana@farmasi.unhas.ac.id

**Number of words: 3026 (Abstract to Conclusion, excluding Figure captions)**
**Number of tables: 1 Table**
**Number of figures: 3 Figures**

**Abstract**

Primaquine (PMQ) is an effective antimalaria drug with several limitations. We report the combinatorial approach of thermoresponsive hydrogels and Dermarollers® for transdermal delivery of PMQ to overcome these limitations. The hydrogels were prepared using Pluronic F127 (PF127) and F68 (PF68). Specifically, HPMC was added into the formulation to improve the bioadhesive properties. Numerous formulations were prepared, showing that formulation comprising 15% PF127, 3% PF68 and 0.4% HPMC with 1% PMQ was selected as the optimum formulation. The formulation showed the gelation temperature around 35°C with bioadhesive strength of 26.43 ± 2.31 dyne.$cm^2$. Importantly, the pH of the formulation was suitable for skin application with the percentage of PMQ recovery of 99.57 ± 3.23%. Moreover, the hydrogels exhibited free-flow liquid at storage and room temperature and high viscosities in the skin temperature. *In vitro* release experiments showed that the release of PMQ was sustained for 24 h. Evaluated in extensive *ex vivo* studies, the treatment with Dermarollers® improved the skin permeation and retention of PMQ for 3 days. In combination with Dermarollers®, the *ex vivo* permeation of PMQ was sustained and the localization of PMQ in the skin was improved over 72 h.

**Keywords:** Primaquine, thermoresponsive, transdermal, Dermarollers®

## 1. Introduction

Around 228 million people in the world were affected by malaria in 2018 [1]. To overcome this, significant improvements have been made to decrease malaria-associated morbidity and death [2,3]. Primaquine (PMQ) has been established as an effective antimalaria drug [4]. Nevertheless, the effectiveness of PMQ is hindered by various problems. Following the administration orally, PMQ experiences first-pass metabolism in the liver, forming carboxyprimaquine (cPQ) through oxidative deamination. This metabolite has been found to show less antimalarial effect and high haemotoxic effect. Additionally, other side effects have been also reported after this administration, including abdominal pain, bitter taste, nausea and long therapy duration [4,5].

Being an attractive route, transdermal delivery has been a promising alternative to oral administration. The delivery of substances transdermally exhibits numerous benefits, namely avoiding first-pass metabolism, painless, excellent compliance and easy accessible [6–8]. Accordingly, this route could be considered as an alternative route to deliver PMQ. However, because of the specific compositions comprised in the skin, the delivery of drugs through this route is challenging [8]. The presence of lipophilic layer, *stratum corneum* (SC), has become the foremost physical blockade for drug permeated through the skin [9]. Moreover, PMQ shows low permeability. Microneedles are alternative devices for transdermal delivery which can disturb SC and create pores, enabling the delivery of drugs to the skin [10–12]. Dermarollers® are one of sorts of microneedles, belonging to solid microneedle type, which have been widely utilized in the cosmetic applications [9,13]. A plethora of research explored the effectiveness of Dermarollers® to enhance the pharmacology effect of numerous active pharmaceutical ingridients [13–15].

It is also important to consider the dosage form of PMQ for this purpose. The poor adherence of the patient is challenge in the malaria therapy. Accordingly, it is important to develop a delivery system that can sustain the release of PMQ through the skin. Thermoresponsive hydrogels are able to turn from a liquid to a gel when moved from the room temperature to the body temperature [16]. This system has been extensively applied as in situ forming vehicle because the sustain release behavior. Several polymers have been explored to prepare this approach. Amongst numerous polymers, in this study, Pluronic® was used as the thermoresponsive agent [17]. The properties of this polymer are sensitive to temperatures, making it suitable for the development of thermosensitive hydrogels with various applications. Importantly, Pluronic® is compatible with several type of cells and biological fluids, as well as non-irritant [16,18,19]. To improve its effectiveness, bioadhesive agents can be included into thermoresponsive formulations. Hydroxy propyl methyl cellulose (HPMC) has been recognized to show this property [20]. It has been reported that, combined with microneedles, this system has successfully improved the penetrability of fluorescein sodium [21]. However, there has been no study reported using the therapeutic agents. Therefore, in this study, for the first time, we developed thermoresponsive hydrogel formulations with bioadhesive properties containing PMQ. Here, we reported the improved transdermal delivery of PMQ using the combination of thermoresponsive and Dermarollers®. Not only that, the deposition of PMQ in the skin was also improved by this combinatorial approach.

## 2. Material and methods

*2.1 Materials*

Primaquine biphospate (PMQ) (purity, ≥98%) was purchased from Sigma Aldrich (Singapore). Pluronic® F68 (PF68) and Pluronic® F127 (PF127) were kindly gifted by BASF SE (Jakarta, Indonesia). Solid microneedles (Dermarollers®) were obtained from SQY® (Guangdong, China).

*2.2 Formulation of thermoresponsive in situ gel of primaquine*

The cold method was used to fabricate the thermoresponsive hydrogels [22]. The composition of the hydrogels is depicted in Table 1. Initially, PF68 and PF127 were dissolved in cold distilled water (4°C) using constant mixing until forming clear solution. Afterwards, HPMC and PMQ were added into the polymeric solution and stored in the refrigerator, forming clear solutions.

**Table 1.** The composition of thermoresponsive hydrogels containing PMQ (% w/v)

| Formula | PMQ | PF127 | PF68 | HPMC |
|---|---|---|---|---|
| **F1** | 1 | 18 | - | - |
| **F2** | 1 | 16 | 2 | - |
| **F3** | 1 | 15 | 3 | - |
| **F4** | 1 | 14 | 4 | - |
| **F5** | 1 | 10 | 8 | - |
| **F6** | 1 | 15 | 3 | 0.20 |
| **F7** | 1 | 15 | 3 | 0.40 |
| **F8** | 1 | 15 | 3 | 0.60 |
| **F9** | 1 | 15 | 3 | 0.80 |
| **F10** | 1 | 15 | 3 | 1 |

*2.3 Determination of gelation temperature and bioadhesive properties*

A test tube inverting technique in a water bath was applied to determine the gelation temperature of the formulation [23]. The tube containing the formulation was placed at 20°C. The temperature was steadily increased until 55°C. The gelation temperature was denoted when the solution turned into a gel after turning the tube over to 90° for 30 s**.** To determine the bioadhesion strength in skin tissue, a modified physical balance was applied [16]. In this study, an abdomen part of rats' skin was used. The skin was shaved and washed using distilled water. Prior to the experiment, the formulations were placed at 37°C for 10 minutes and then applied to the skin attached to the balance in one side. Afterward, weights were placed in the other side of the balance until the formulations were detached from the skin. Finally, the bioadhesive strength was determined using the following calculation:

$$\text{Bioadhesive strength} = \frac{weight\ (g) x\ 100}{surface\ area\ (cm^2)} \text{ x } 9.81$$

*2.4 Determination of pH, viscosity and rheological behavior*

The pH of the hydrogel formulation was determined using pH meter. The viscosity and rheology thermoresponsive gel were measured using a DV-III viscometer (RV model, Brookfield, USA). For viscosity measurement, gels were tested at cold temperature (4°C), room temperature (25°C) and skin temperature (32°C).

*2.6 Drug content analysis and in vitro release study of PMQ*

Drug content of PMQ was assessed using spectrophotometer UV-Vis at 265 nm. The *in vitro* release of PMQ from thermoresponsive hydrogel was carried out using membraneless dissolution method using phosphate-buffered saline (PBS) as release media. Briefly, 10 g of hydrogel was placed into orbital shaker at 37 ± 0.5 °C. After the formulation became gel, PBS (5 mL) was added into the gels. At predetermined time (0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 12 h and 24 h), 1 mL of medium was taken and replaced with fresh medium. The concentration of PMQ was assessed using spectrophotometer UV-Vis at 265 nm.

*2.8 Ex vivo skin permeation and retention study of PMQ*

The *ex vivo* permeation of PMQ from thermoresporesponsive hydrogels was performed through rats' skin using vertical Franz diffusion cell with PBS as media. The skin was placed between donor and receiver compartment of Franz diffusion cell. The diffusion cell was stirred at 100 rpm and the temperature was maintained at 37 ± 0.5 °C. The formulation (equal 10 mg of PMQ) was placed into donor compartment. With total time of 72 h, at predetermined time, 0.5 mL of permeation media was withdrawn and replaced with new media. In this study, the effect of skin pretreatment with various length of Dermarollers®, namely 0.5 mm, 1 mm and 1.5 mm, was evaluated. Moreover, the concentration of

PMQ retained in the skin was determined by extracting PMQ from the skin using methanol and analyzed using spectrophotometer UV-Vis at 265 nm.

## 3. Results and discussion

*3.1 Preparation of primaquine-loaded thermoresponsive in situ gel*

The main purpose of this study was to overcome the delivery issue of PMQ in the malaria treatment. As discussed earlier, to sustain the release of PMQ transdermally, thermoresponsive hydrogels were developed. Pluronics were selected as the main polymer to form this approach. The representative images of the formulation are depicted in Figure 1A and 1B.

These compounds are triblock copolymers containing poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) (PEO-PPO-PEO) compositions. Due to the presence of PEO as hydrophilic part and PPO as hydrophobic part, these polymers possess amphiphilic characteristics. At low temperature, Pluronics are the form of solution because they are in the unimers form. When the temperature increases, the unimers connects each other, forming micelles and turning into semisolid form. Here, we evaluated different concentration of PF127 and PF68 to obtain the suitable gelation temperature. The addition PF68 into PF127 has been found to show numerous advantages, particularly in improving the low gelation temperature of PF127 [19]. Several studies have combined other types of Pluronics into PF127 to improve the thermosenstivie properties of the polymers [24,25]. Despite several benefits, it was found that Pluronics do not possess adequate bioadhesive properties [19]. Accordingly, to improve the adhesion to the skin, HPMC was used. HPMC has been reported to form an adequate hydroxyl interaction with biological membrane, resulting in the strong bioadhesive property [18].

*3.2 Determination of gelation temperature measurement*

The ration of PPO/PEO of pluronics have been reported to affect the gelation temperature of the hydrogels. The hydrophobicity of PPO could decrease the gelation temperature, where the hydrophilicity of PEO could increase the gelation temperature. As PF127 contains more PPO than PF68, as presented in Figure 1A, the higher concentration of PF68 resulted in lower gelation temperature. The use of PF127 alone resulted in the gelation temperature below the body temperature. This phenomenon was also shown in other studies [18,24,25], indicating the need of combining PF127 with other type of Pluronics. Our results showed that F3 containing 15% PF127 and 3% PF68 showed the gelation temperature around body temperature and, therefore, this ratio was investigated to evaluate the effect of HPMC as bioadhesive agent. As shown in Figure 1C, above 0.4%, the formulations possessed low gelation temperature (<32$^o$C), making the inappropriate for thermoresponsive preparations. Therefore, it was crucial to consider the consider the bioadhesive agent in the thermoresponsive preparations. It was important to ensure that the addition of bioadhesive compounds did not alter the thermosensitive properties of the preparations.

*3.3 Determination of bioadhesion strength*

To improve the adhesion with the desired site of action, hydrophilic polymers, HPMC, was added into the formulation. Figure 1D shows that the bioadhesion strength of the hydrogels to the skin was improved following the increase on HPMC concentration. Interestingly, there were no significant differences ($p > 0.05$) in bioadhesive strength when HPMC used in the concentration of 0.4% compared to 0.6% and 08%. Although 1% of HPMC showed significant improvement ($p < 0.05$) in bioadhesive properties, the formulation did not meet the requirement for thermosensitive preparations. In addition to skin delivery, HPMC has been widely used as bioadhesive agent for several application, including vaginal, ocular and oral [18,19,26], showing that the incorporation of HPMC in pharmaceutical dosage forms could potentially improve the bioadhesive properties the preparation in various biological membrane.

*3.4 Determination of pH, viscosity and rheological behavior*

It was crucial to ensure that the application of the hydrogels did not produce possible irritation to the skin. This could be achieved by preparing the formulation having pH around to the skin pH (± 5.8) [20]. The results exhibited that all hydrogels possessed pH values tolerated by the skin (Figure 2A). Therefore, the application of the thermoresponsive hydrogels could not potentially cause any irritation of the skin.

The desired hydrogels were those which showed free-flow liquid with low viscosities at storage and room temperature and high viscosities in semisolid form in the skin temperature. Figure 2B depicts that the ratio of PF127 and PF168 affected the viscosities of the hydrogels. Higher concentration of PF127 showed higher viscosity due to higher amount of triblock chains and, therefore, resulted in higher micelle size [25]. Showing similar trend with gelation temperature, the use of HPMC increased the viscosities of the hydrogels at all temperatures. As presented in Figure 2B, only formulations containing HPMC with the concentrations below 0.6% showed lower viscosities at room temperatures. Accordingly, F7 (0.4% HPMC) was considered as the optimum formulation with appropriate properties for the thermoresponsive preparations. With respect to rheological properties, all formulations showed shear thinning behavior showing low viscosity in the high rate of share (Figure 2C). These results were in good agreement with the gelation temperature determination, showing that the formulations with low gelation temperature exhibited higher viscosities at all temperature tested.

### *3.5 Drug content analysis and in vitro release study of PMQ*

The concentration of PMQ in all formulations were between 98-100% (Figure 2D). This indicated that the formulation process did not influence the concentration of PMQ in the formulation. It was previously reported that the percentage recovery of dosage forms should be between 95% and 105% [27]. In *in vitro* release, PMQ released exhibited biphasic manner. The *in vitro* release results of all formulations are shown in Figure 3A and 3B. During the first hour, the burst release behavior was observed in all cases which might be due to the presence of PMQ on the surface of the gels. The burst release was previously shown in similar approach using fluorescein sodium as a model drug [21]. Furthermore, the sustained release was observed over 24 h, showing the ability of pluronics to sustain the release. It is important to note that F4 and F5 showing low gelation temperature, due to the liquid form at skin temperature, all PMQ released less than 5 h. Importantly, F7 which contained 0.4% HPMC and showed desirable mucoadhesive and thermosensitive properties, exhibited similar behavior with optimum formulation without HPMC (F3). Accordingly, this formulation was selected for *ex vivo* studies.

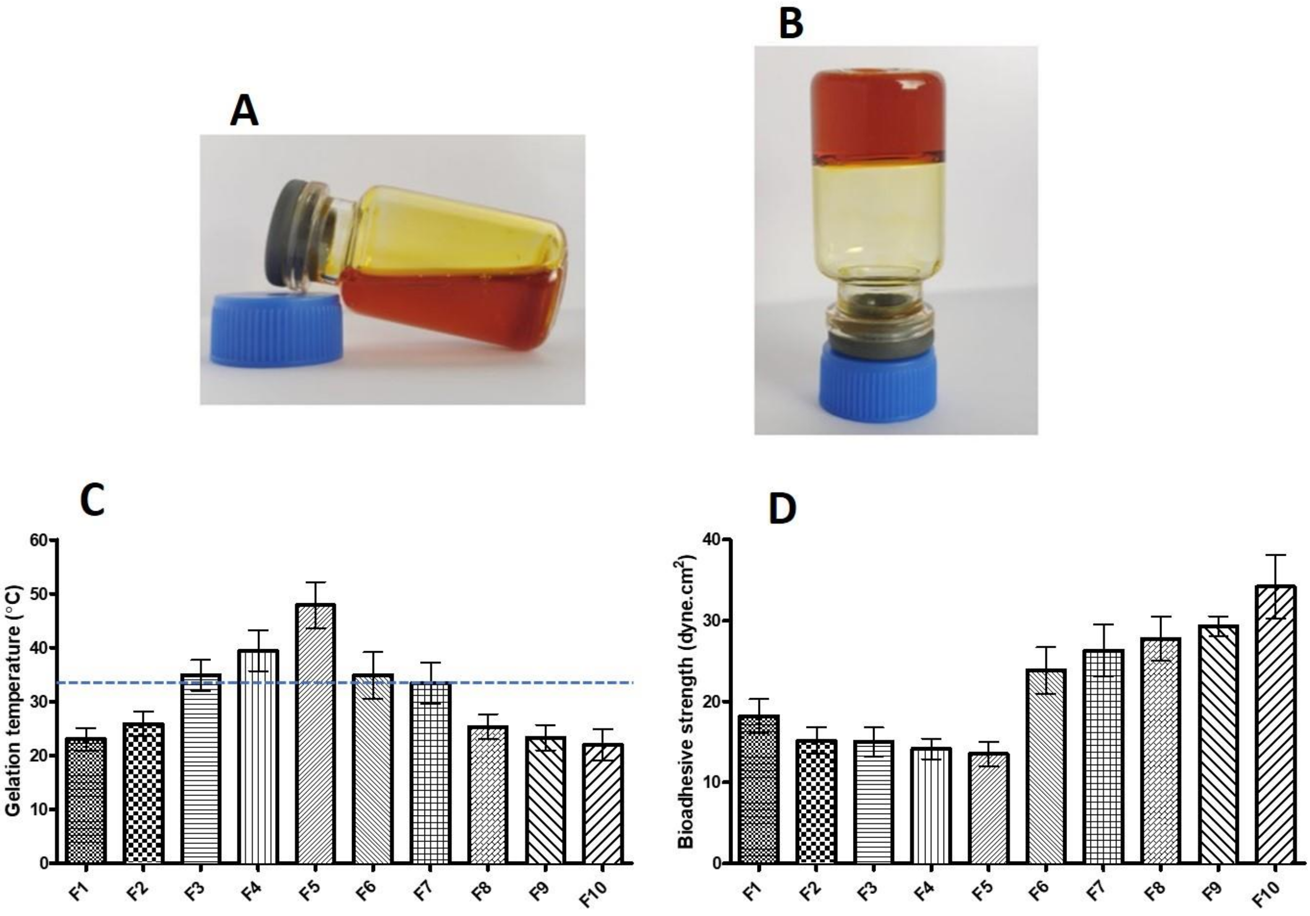


**Figure 1**. Illustrative photograph of the formulation at room temperature (A) and skin temperature (B). The results of the determination of the gelation temperature of thermoresponsive hydrogels containing PMQ (mean ± S.D., *n=3*). The temperature of the skin is indicated by the dash blue line (C). The biooadhesion properties thermoresponsive hydrogels containing PMQ (mean ± S.D., *n=3*) (D).

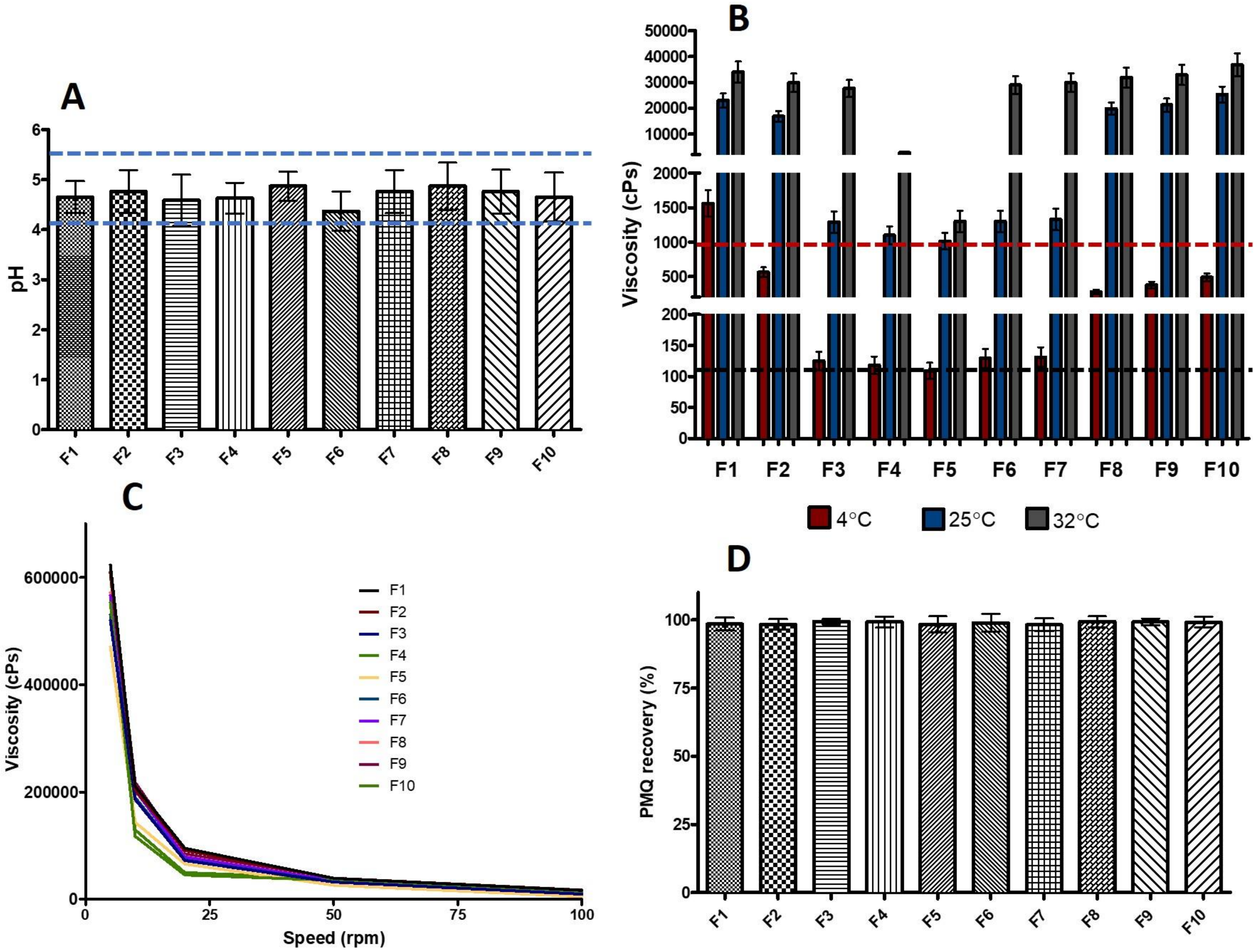


**Figure 2**. The results of the pH determination of thermoresponsive hydrogels containing PMQ (mean ± S.D., *n=3*). The pH tolerated by the skin is indicated by the blue das lines (A). Viscosities of thermoresponsive hydrogels containing PMQ at various temperatures, including 4ºC, 25ºC, and 32ºC (mean ± S.D., *n=3*). The liquid form of the formulations is indicated by the values below black dash line and between black and red lines. The gel formation is indicated by the values above red dash line (B). The rheology pattern of thermoresponsive hydrogels containing PMQ (C). Drug recoveries percentages of thermoresponsive hydrogels containing PMQ (D) (mean ± S.D., *n=3*)

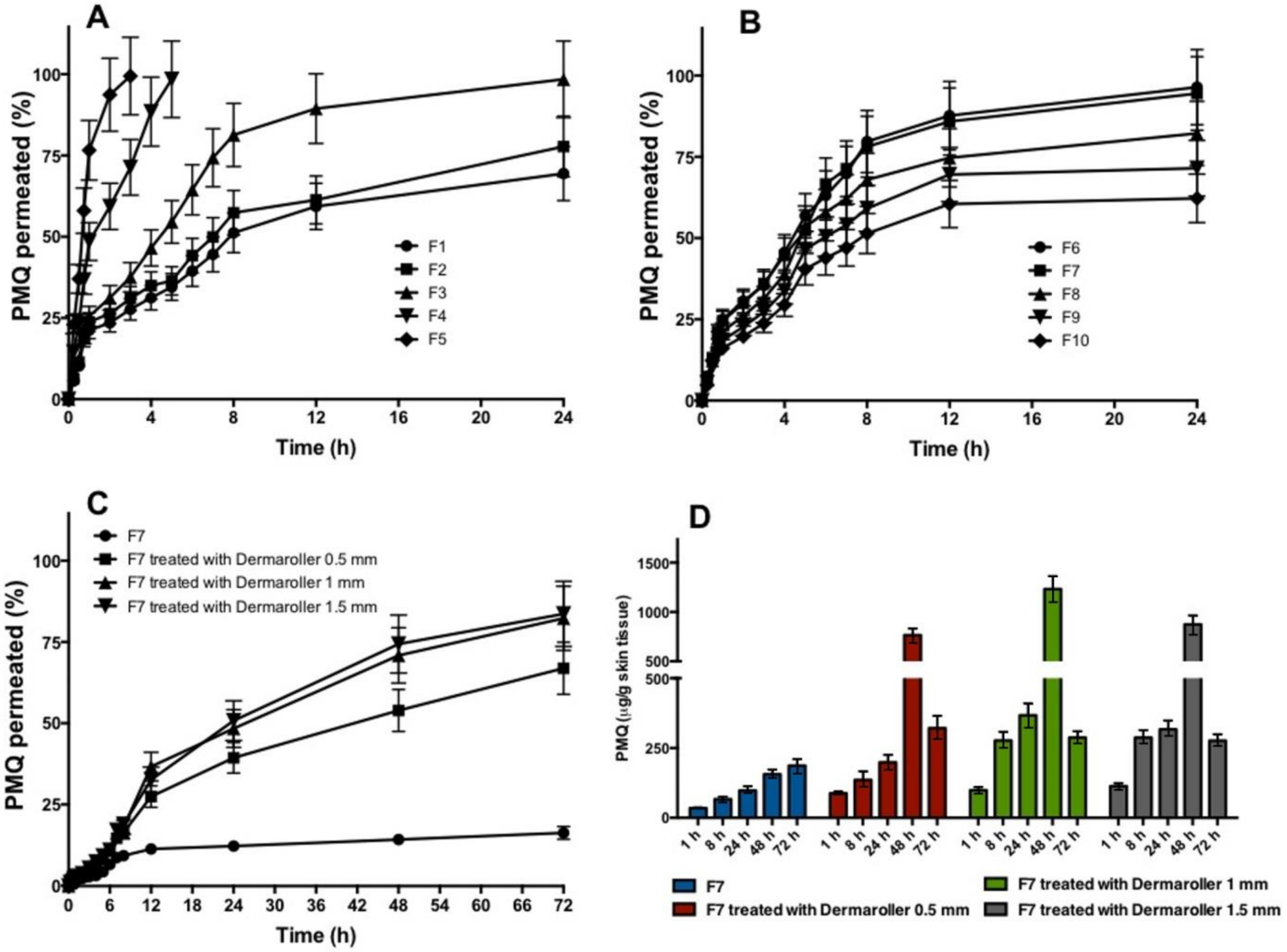


**Figure 3**. *In vitro* release of PMQ from various thermoresponsive hydrogels (A and B) (mean ± S.D., *n=3*). *Ex vivo* skin permeation (C) and skin retention (D) of F7 as optimized formulation following the administration thermoresponsive hydrogels with and without the treatment of Dermarollers® with various length (mean ± S.D., *n=3*)

*3.7 Ex vivo skin permeation and retention study of PMQ*

It was shown that due to the hydrophilicity of PMQ, it was difficult to permeate the lipophilic of SC and the concentration of PMQ permeating the skin was less 25% over 72 h. After the treatment using Dermarollers®, the concentration of PMQ permeating the skin was significantly enhanced with more than 50% for 0.5 mm Dermarollers® and more than 75% for Dermarollers® 1 mm and 1.5 mm. This was because the micropore created in the skin by Dermarollers®, disrupting the physical barrier of SC. It was important to note that sustained release behavior was observed over 72 h in all types of Dermarollers®. Interestingly, in *ex vivo* retention studies, it was found that the depositions of PMQ were significantly higher ($p < 0.05$) after the treatment using Dermarollers®. Considering non-significant different between

1 mm and 1.5 mm Dermarollers®, 1 mm was considered as the optimum length as the use of long needle could potentially cause discomfort of patient following its application. In our study, for the first time, it was concluded that the delivery of PMQ could be controlled using thermoresponsive hydrogels and Dermarollers®, providing sustained release permeation manner over 72 h, where PMQ was well-deposited in the skin and were released from thermoresponsive hydrogels. Several studies have investigated the administration of Dermarollers® to enhance the skin permeation of different drugs [13,14,28], showing the improvement of transdermal delivery in comparison with the conventional delivery systems. Previously, PMQ was developed in nanoemulsion delivery system [4]. It was reported that the nanoemulsion could improve and control the transdermal delivery for 24 h. In our study, the sustained release profile was maintained over 72 h. This was due to the formation of *in situ* depot from thermoresponsive system which allowed the improvement of control release pattern, indicating the inventiveness of our approach. However, before this approach can applied in the clinical treatment, several considerations are required. The sterility of this system should be considered. With regard to this, previous study has shown that the penetration of microorganism across the epidermis following the application of microneedles was found to be negligible [29]. Therefore, we could assume that the pores created by the use of microneedle should not cause the risk of infection. However, further studies are required to ensure this in the clinical applications. Moreover, the storage stability of the thermoresponsive gel should be investigated. It is also critical to ensure the reproducibility of this combination approach performed by patients. To achieve this, the pressure applied should be similar. Automated dermaroller has been developed to ensure the reproducibility of pressure applied [30], which could be potentially combined with the thermoresponsive developed in this study. Following this promising results, further *in vivo* studies should be carried out to investigate the efficacy, the pharmacokinetic profile and the dose determination of this approach. Moving forward, the impact of the deposition of the polymers should be evaluated. The use of Pluronics-based in the skin administration following microneedle applications is still limited. Therefore, the attention should not only be given to the pharmacokinetic profiles of the drugs, but also to the polymers used, particularly in the clearance profile. Finally, the scale-up manufacturing process should be considered in order to ensure that the method preparation can be applied in the industrial scale.

**4. Conclusion**

In this study, for the first time, we developed thermoresponsive hydrogels with bioadhesive properties to transdermally delivery PMQ combined with Dermarollers®. The combination of PF127 and PF68 with the ratio of 15% and 3% with 0.4% HPMC was found to be the optimal hydrogel with suitable thermoresponsive and mucoadhesive characteristics. The approach could potentially sustain the release of PMQ over 24 h. Essentially, the combination with Dermarollers® was able to enhance and control the *ex vivo* permeation and retention of PMQ through rats' skin over 72 h.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

## AUTHORS CONTRIBUTION

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript

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# Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®

Andi Dian Permana*, Diany Elim, Putri Wulandari Resky Ananda, Hilman Syamami Zaman, Wahdaniyah Muslimin, Muhamad Gilang Ramadhan Tunggeng

**Faculty of Pharmacy, Hasanuddin University, Makassar, 90245, Indonesia**

***Corresponding author:**
Andi Dian Permana
Faculty of Pharmacy, Hasanuddin University, Indonesia
Email: andi.dian.permana@farmasi.unhas.ac.id

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**Abstract**

Primaquine (PMQ) is an effective antimalaria drug with several limitations. We report the combinatorial approach of thermoresponsive hydrogels and Dermarollers® for transdermal delivery of PMQ to overcome these limitations. The hydrogels were prepared using Pluronic F127 (PF127) and F68 (PF68). Specifically, HPMC was added into the formulation to improve the bioadhesive properties. Numerous formulations were prepared, showing that formulation comprising 15% PF127, 3% PF68 and 0.4% HPMC with 1% PMQ was selected as the optimum formulation. The formulation showed the gelation temperature around 35°C with bioadhesive strength of 26.43 ± 2.31 dyne.cm$^2$. Importantly, the pH of the formulation was suitable for skin application with the percentage of PMQ recovery of 99.57 ± 3.23%. Moreover, the hydrogels exhibited free-flow liquid at storage and room temperature and high viscosities in the skin temperature. *In vitro* release experiments showed that the release of PMQ was sustained for 24 h. Evaluated in extensive *ex vivo* studies, the treatment with Dermarollers® improved the skin permeation and retention of PMQ for 3 days. In combination with Dermarollers®, the *ex vivo* permeation of PMQ was sustained and the localization of PMQ in the skin was improved over 72 h.

**Keywords:** Primaquine, thermoresponsive, transdermal, Dermarollers®

## 1. Introduction

Around 228 million people in the world were affected by malaria in 2018 [1]. To overcome this, significant improvements have been made to decrease malaria-associated morbidity and death [2,3]. Primaquine (PMQ) has been established as an effective antimalaria drug [4]. Nevertheless, the effectiveness of PMQ is hindered by various problems. Following the administration orally, PMQ experiences first-pass metabolism in the liver, forming carboxyprimaquine (cPQ) through oxidative deamination. This metabolite has been found to show less antimalarial effect and high haemotoxic effect. Additionally, other side effects have been also reported after this administration, including abdominal pain, bitter taste, nausea and long therapy duration [4,5].

Being an attractive route, transdermal delivery has been a promising alternative to oral administration. The delivery of substances transdermally exhibits numerous benefits, namely avoiding first-pass metabolism, painless, excellent compliance and easy accessible [6–8]. Accordingly, this route could be considered as an alternative route to deliver PMQ. However, because of the specific compositions comprised in the skin, the delivery of drugs through this route is challenging [8]. The presence of lipophilic layer, *stratum corneum* (SC), has become the foremost physical blockade for drug permeated through the skin [9]. Moreover, PMQ shows low permeability. Microneedles are alternative devices for transdermal delivery which can disturb SC and create pores, enabling the delivery of drugs to the skin [10–12]. Dermarollers® are one of sorts of microneedles, belonging to solid microneedle type, which have been widely utilized in the cosmetic applications [9,13]. A plethora of research explored the effectiveness of Dermarollers® to enhance the pharmacology effect of numerous active pharmaceutical ingridients [13–15].

It is also important to consider the dosage form of PMQ for this purpose. The poor adherence of the patient is challenge in the malaria therapy. Accordingly, it is important to develop a delivery system that can sustain the release of PMQ through the skin. Thermoresponsive hydrogels are able to turn from a liquid to a gel when moved from the room temperature to the body temperature [16]. This system has been extensively applied as in situ forming vehicle because the sustain release behavior. Several polymers have been explored to prepare this approach. Amongst numerous polymers, in this study, Pluronic® was used as the thermoresponsive agent [17]. The properties of this polymer are sensitive to temperatures, making it suitable for the development of thermosensitive hydrogels with various applications. Importantly, Pluronic® is compatible with several type of cells and biological fluids, as well as non-irritant [16,18,19]. To improve its effectiveness, bioadhesive agents can be included into thermoresponsive formulations. Hydroxy propyl methyl cellulose (HPMC) has been recognized to show this property [20]. It has been reported that, combined with microneedles, this system has successfully improved the penetrability of fluorescein sodium [21]. However, there has been no study reported using the therapeutic agents. Therefore, in this study, for the first time, we developed thermoresponsive hydrogel formulations with bioadhesive properties containing PMQ. Here, we reported the improved transdermal delivery of PMQ using the combination of thermoresponsive and Dermarollers®. Not only that, the deposition of PMQ in the skin was also improved by this combinatorial approach.

## 2. Material and methods

*2.1 Materials*

Primaquine biphospate (PMQ) (purity, ≥98%) was purchased from Sigma Aldrich (Singapore). Pluronic® F68 (PF68) and Pluronic® F127 (PF127) were kindly gifted by BASF SE (Jakarta, Indonesia). Solid microneedles (Dermarollers®) were obtained from SQY® (Guangdong, China).

*2.2 Formulation of thermoresponsive in situ gel of primaquine*

The cold method was used to fabricate the thermoresponsive hydrogels [22]. The composition of the hydrogels is depicted in Table 1. Initially, PF68 and PF127 were dissolved in cold distilled water (4°C) using constant mixing until forming clear solution. Afterwards, HPMC and PMQ were added into the polymeric solution and stored in the refrigerator, forming clear solutions.

**Table 1.** The composition of thermoresponsive hydrogels containing PMQ (% w/v)

| Formula | PMQ | PF127 | PF68 | HPMC |
|---|---|---|---|---|
| **F1** | 1 | 18 | - | - |
| **F2** | 1 | 16 | 2 | - |
| **F3** | 1 | 15 | 3 | - |
| **F4** | 1 | 14 | 4 | - |
| **F5** | 1 | 10 | 8 | - |
| **F6** | 1 | 15 | 3 | 0.20 |
| **F7** | 1 | 15 | 3 | 0.40 |
| **F8** | 1 | 15 | 3 | 0.60 |
| **F9** | 1 | 15 | 3 | 0.80 |
| **F10** | 1 | 15 | 3 | 1 |

*2.3 Determination of gelation temperature and bioadhesive properties*

A test tube inverting technique in a water bath was applied to determine the gelation temperature of the formulation [23]. The tube containing the formulation was placed at 20°C. The temperature was steadily increased until 55°C. The gelation temperature was denoted when the solution turned into a gel after turning the tube over to 90° for 30 s**.** To determine the bioadhesion strength in skin tissue, a modified physical balance was applied [16]. In this study, an abdomen part of rats' skin was used. The skin was shaved and washed using distilled water. Prior to the experiment, the formulations were placed at 37°C for 10 minutes and then applied to the skin attached to the balance in one side. Afterward, weights were placed in the other side of the balance until the formulations were detached from the skin. Finally, the bioadhesive strength was determined using the following calculation:

$$\text{Bioadhesive strength} = \frac{weight\ (g) x\ 100}{surface\ area\ (cm^{2})} \text{ x } 9.81$$

*2.4 Determination of pH, viscosity and rheological behavior*

The pH of the hydrogel formulation was determined using pH meter. The viscosity and rheology thermoresponsive gel were measured using a DV-III viscometer (RV model, Brookfield, USA). For viscosity measurement, gels were tested at cold temperature (4°C), room temperature (25°C) and skin temperature (32°C).

*2.6 Drug content analysis and in vitro release study of PMQ*

Drug content of PMQ was assessed using spectrophotometer UV-Vis at 265 nm. The *in vitro* release of PMQ from thermoresponsive hydrogel was carried out using membraneless dissolution method using phosphate-buffered saline (PBS) as release media. Briefly, 10 g of hydrogel was placed into orbital shaker at 37 ± 0.5 °C. After the formulation became gel, PBS (5 mL) was added into the gels. At predetermined time (0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 12 h and 24 h), 1 mL of medium was taken and replaced with fresh medium. The concentration of PMQ was assessed using spectrophotometer UV-Vis at 265 nm.

*2.8 Ex vivo skin permeation and retention study of PMQ*

The *ex vivo* permeation of PMQ from thermoresporesponsive hydrogels was performed through rats' skin using vertical Franz diffusion cell with PBS as media. The skin was placed between donor and receiver compartment of Franz diffusion cell. The diffusion cell was stirred at 100 rpm and the temperature was maintained at 37 ± 0.5 °C. The formulation (equal 10 mg of PMQ) was placed into donor compartment. With total time of 72 h, at predetermined time, 0.5 mL of permeation media was withdrawn and replaced with new media. In this study, the effect of skin pretreatment with various length of Dermarollers®, namely 0.5 mm, 1 mm and 1.5 mm, was evaluated. Moreover, the concentration of

PMQ retained in the skin was determined by extracting PMQ from the skin using methanol and analyzed using spectrophotometer UV-Vis at 265 nm.

## 3. Results and discussion

### *3.1 Preparation of primaquine-loaded thermoresponsive in situ gel*

The main purpose of this study was to overcome the delivery issue of PMQ in the malaria treatment. As discussed earlier, to sustain the release of PMQ transdermally, thermoresponsive hydrogels were developed. Pluronics were selected as the main polymer to form this approach. The representative images of the formulation are depicted in Figure 1A and 1B.

These compounds are triblock copolymers containing poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) (PEO-PPO-PEO) compositions. Due to the presence of PEO as hydrophilic part and PPO as hydrophobic part, these polymers possess amphiphilic characteristics. At low temperature, Pluronics are the form of solution because they are in the unimers form. When the temperature increases, the unimers connects each other, forming micelles and turning into semisolid form. Here, we evaluated different concentration of PF127 and PF68 to obtain the suitable gelation temperature. The addition PF68 into PF127 has been found to show numerous advantages, particularly in improving the low gelation temperature of PF127 [19]. Several studies have combined other types of Pluronics into PF127 to improve the thermosenstivie properties of the polymers [24,25]. Despite several benefits, it was found that Pluronics do not possess adequate bioadhesive properties [19]. Accordingly, to improve the adhesion to the skin, HPMC was used. HPMC has been reported to form an adequate hydroxyl interaction with biological membrane, resulting in the strong bioadhesive property [18].

### *3.2 Determination of gelation temperature measurement*

The ration of PPO/PEO of pluronics have been reported to affect the gelation temperature of the hydrogels. The hydrophobicity of PPO could decrease the gelation temperature, where the hydrophilicity of PEO could increase the gelation temperature. As PF127 contains more PPO than PF68, as presented in Figure 1A, the higher concentration of PF68 resulted in lower gelation temperature. The use of PF127 alone resulted in the gelation temperature below the body temperature. This phenomenon was also shown in other studies [18,24,25], indicating the need of combining PF127 with other type of Pluronics. Our results showed that F3 containing 15% PF127 and 3% PF68 showed the gelation temperature around body temperature and, therefore, this ratio was investigated to evaluate the effect of HPMC as bioadhesive agent. As shown in Figure 1C, above 0.4%, the formulations possessed low gelation temperature (<32$^{o}$C), making the inappropriate for thermoresponsive preparations. Therefore, it was crucial to consider the consider the bioadhesive agent in the thermoresponsive preparations. It was important to ensure that the addition of bioadhesive compounds did not alter the thermosensitive properties of the preparations.

*3.3 Determination of bioadhesion strength*

To improve the adhesion with the desired site of action, hydrophilic polymers, HPMC, was added into the formulation. Figure 1D shows that the bioadhesion strength of the hydrogels to the skin was improved following the increase on HPMC concentration. Interestingly, there were no significant differences ($p > 0.05$) in bioadhesive strength when HPMC used in the concentration of 0.4% compared to 0.6% and 08%. Although 1% of HPMC showed significant improvement ($p < 0.05$) in bioadhesive properties, the formulation did not meet the requirement for thermosensitive preparations. In addition to skin delivery, HPMC has been widely used as bioadhesive agent for several application, including vaginal, ocular and oral [18,19,26], showing that the incorporation of HPMC in pharmaceutical dosage forms could potentially improve the bioadhesive properties the preparation in various biological membrane.

*3.4 Determination of pH, viscosity and rheological behavior*

It was crucial to ensure that the application of the hydrogels did not produce possible irritation to the skin. This could be achieved by preparing the formulation having pH around to the skin pH (± 5.8) [20]. The results exhibited that all hydrogels possessed pH values tolerated by the skin (Figure 2A). Therefore, the application of the thermoresponsive hydrogels could not potentially cause any irritation of the skin.

The desired hydrogels were those which showed free-flow liquid with low viscosities at storage and room temperature and high viscosities in semisolid form in the skin temperature. Figure 2B depicts that the ratio of PF127 and PF168 affected the viscosities of the hydrogels. Higher concentration of PF127 showed higher viscosity due to higher amount of triblock chains and, therefore, resulted in higher micelle size [25]. Showing similar trend with gelation temperature, the use of HPMC increased the viscosities of the hydrogels at all temperatures. As presented in Figure 2B, only formulations containing HPMC with the concentrations below 0.6% showed lower viscosities at room temperatures. Accordingly, F7 (0.4% HPMC) was considered as the optimum formulation with appropriate properties for the thermoresponsive preparations. With respect to rheological properties, all formulations showed shear thinning behavior showing low viscosity in the high rate of share (Figure 2C). These results were in good agreement with the gelation temperature determination, showing that the formulations with low gelation temperature exhibited higher viscosities at all temperature tested.

*3.5 Drug content analysis and in vitro release study of PMQ*

The concentration of PMQ in all formulations were between 98-100% (Figure 2D). This indicated that the formulation process did not influence the concentration of PMQ in the formulation. It was previously reported that the percentage recovery of dosage forms should be between 95% and 105% [27]. In *in vitro* release, PMQ released exhibited biphasic manner. The *in vitro* release results of all formulations are shown in Figure 3A and 3B. During the first hour, the burst release behavior was observed in all cases which might be due to the presence of PMQ on the surface of the gels. The burst release was previously shown in similar approach using fluorescein sodium as a model drug [21]. Furthermore, the sustained release was observed over 24 h, showing the ability of pluronics to sustain the release. It is important to note that F4 and F5 showing low gelation temperature, due to the liquid form at skin temperature, all PMQ released less than 5 h. Importantly, F7 which contained 0.4% HPMC and showed desirable mucoadhesive and thermosensitive properties, exhibited similar behavior with optimum formulation without HPMC (F3). Accordingly, this formulation was selected for *ex vivo* studies.

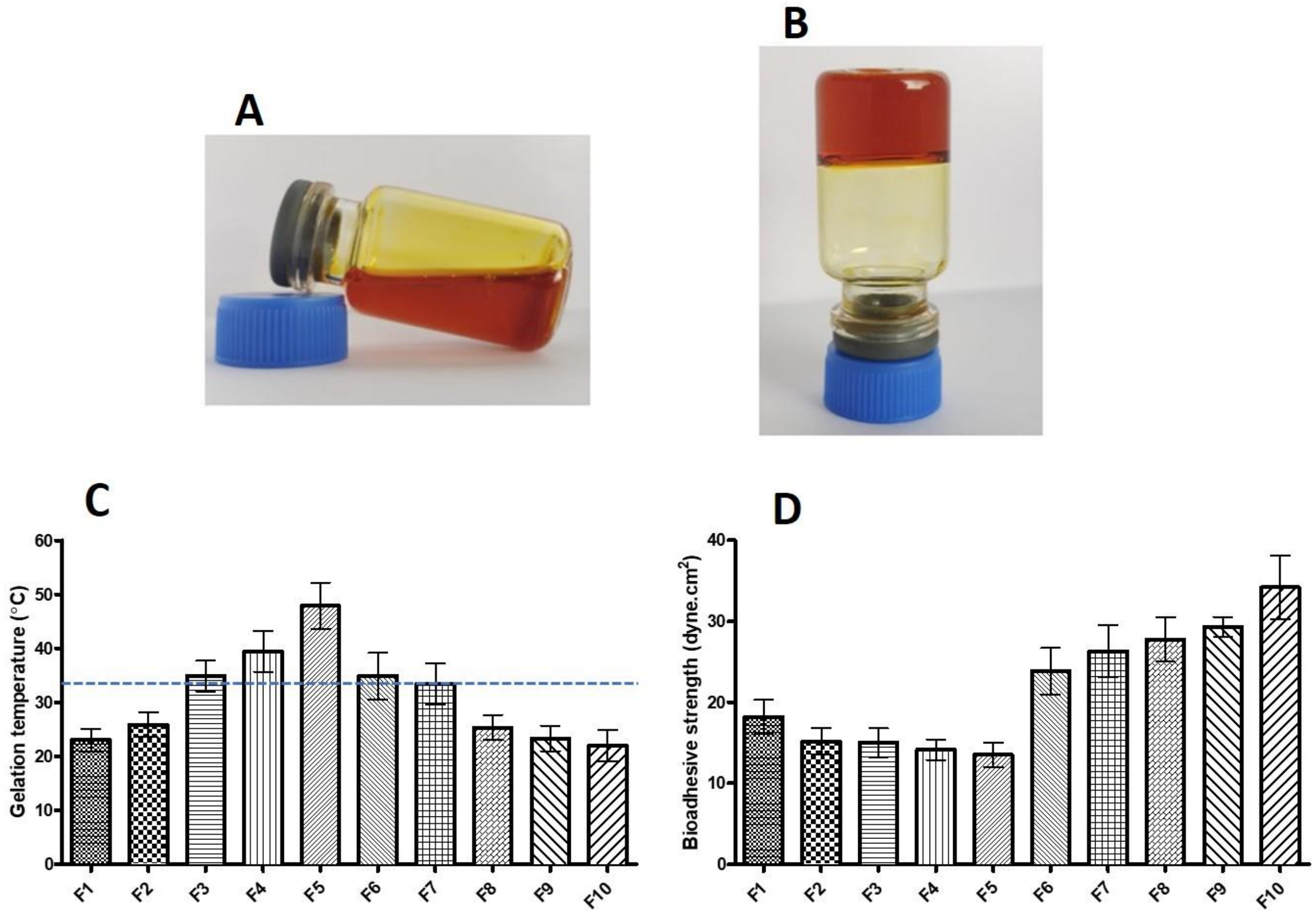


**Figure 1**. Illustrative photograph of the formulation at room temperature (A) and skin temperature (B). The results of the determination of the gelation temperature of thermoresponsive hydrogels containing PMQ (mean ± S.D., *n=3*). The temperature of the skin is indicated by the dash blue line (C). The biooadhesion properties thermoresponsive hydrogels containing PMQ (mean ± S.D., *n=3*) (D).

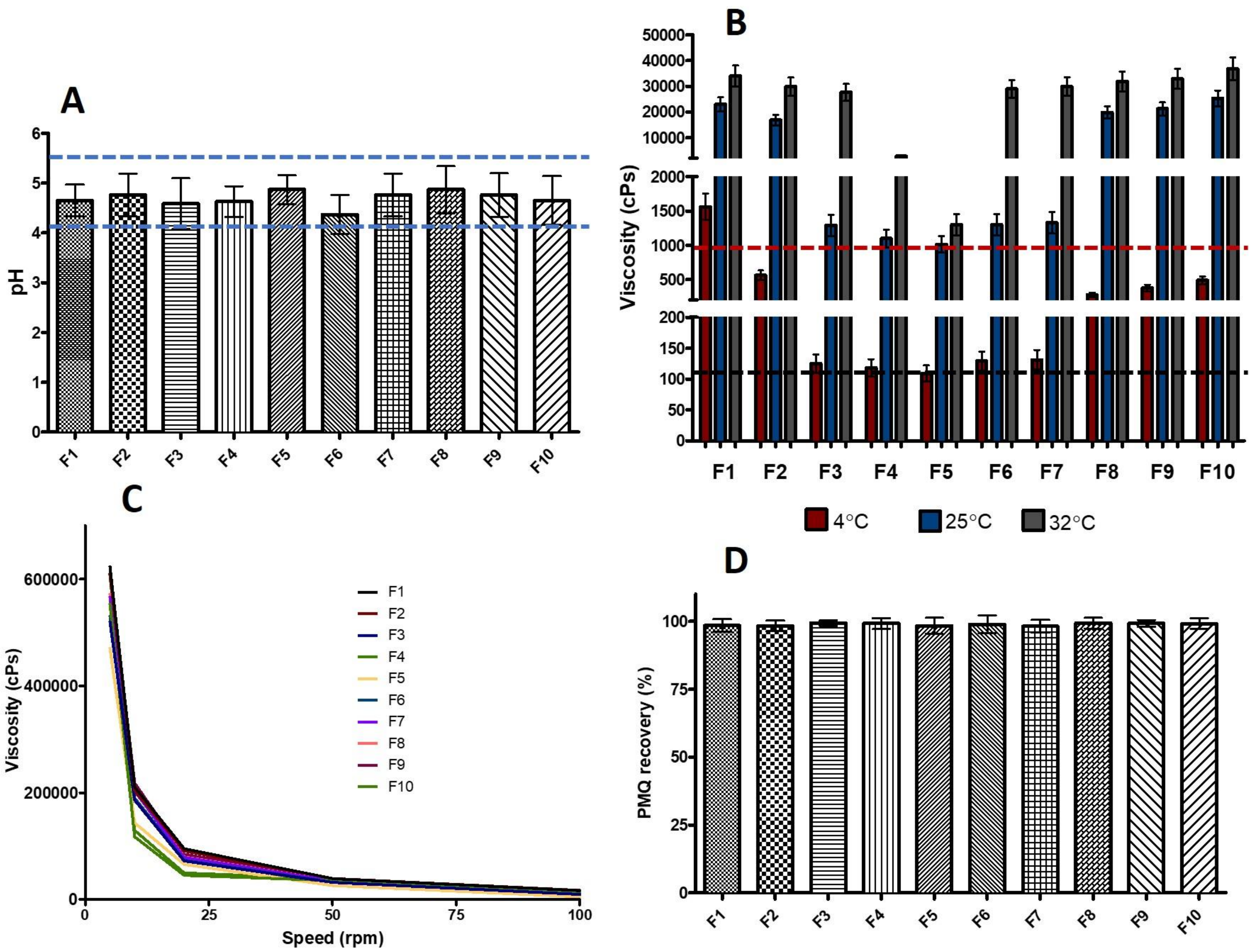


**Figure 2**. The results of the pH determination of thermoresponsive hydrogels containing PMQ (mean ± S.D., *n=3*). The pH tolerated by the skin is indicated by the blue das lines (A). Viscosities of thermoresponsive hydrogels containing PMQ at various temperatures, including 4ºC, 25ºC, and 32ºC (mean ± S.D., *n=3*). The liquid form of the formulations is indicated by the values below black dash line and between black and red lines. The gel formation is indicated by the values above red dash line (B). The rheology pattern of thermoresponsive hydrogels containing PMQ (C). Drug recoveries percentages of thermoresponsive hydrogels containing PMQ (D) (mean ± S.D., *n=3*)

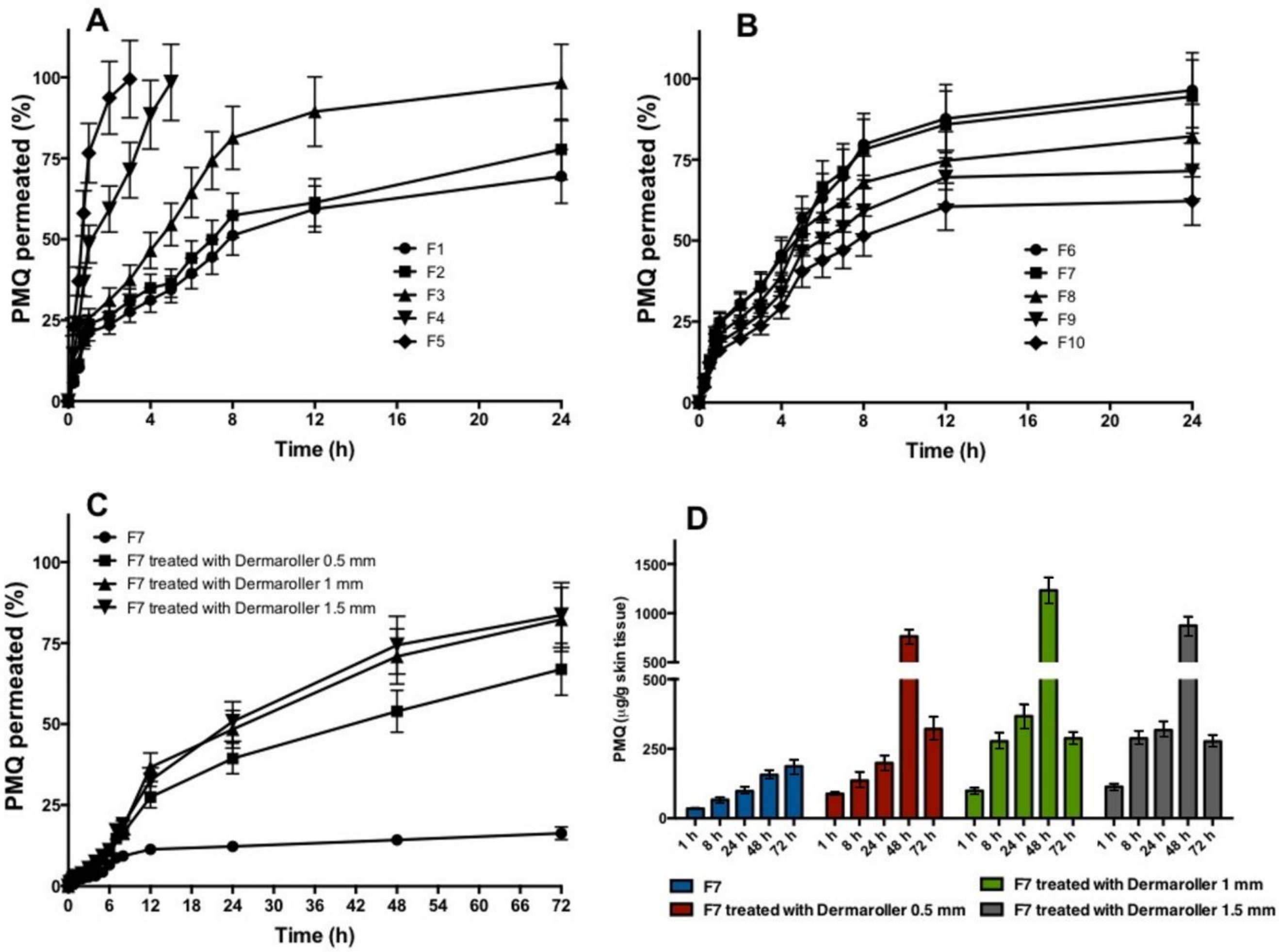


**Figure 3**. *In vitro* release of PMQ from various thermoresponsive hydrogels (A and B) (mean ± S.D., *n=3*). *Ex vivo* skin permeation (C) and skin retention (D) of F7 as optimized formulation following the administration thermoresponsive hydrogels with and without the treatment of Dermarollers® with various length (mean ± S.D., *n=3*)

*3.7 Ex vivo skin permeation and retention study of PMQ*

It was shown that due to the hydrophilicity of PMQ, it was difficult to permeate the lipophilic of SC and the concentration of PMQ permeating the skin was less 25% over 72 h. After the treatment using Dermarollers®, the concentration of PMQ permeating the skin was significantly enhanced with more than 50% for 0.5 mm Dermarollers® and more than 75% for Dermarollers® 1 mm and 1.5 mm. This was because the micropore created in the skin by Dermarollers®, disrupting the physical barrier of SC. It was important to note that sustained release behavior was observed over 72 h in all types of Dermarollers®. Interestingly, in *ex vivo* retention studies, it was found that the depositions of PMQ were significantly higher ($p < 0.05$) after the treatment using Dermarollers®. Considering non-significant different between

1 mm and 1.5 mm Dermarollers®, 1 mm was considered as the optimum length as the use of long needle could potentially cause discomfort of patient following its application. In our study, for the first time, it was concluded that the delivery of PMQ could be controlled using thermoresponsive hydrogels and Dermarollers®, providing sustained release permeation manner over 72 h, where PMQ was well-deposited in the skin and were released from thermoresponsive hydrogels. Several studies have investigated the administration of Dermarollers® to enhance the skin permeation of different drugs [13,14,28], showing the improvement of transdermal delivery in comparison with the conventional delivery systems. Previously, PMQ was developed in nanoemulsion delivery system [4]. It was reported that the nanoemulsion could improve and control the transdermal delivery for 24 h. In our study, the sustained release profile was maintained over 72 h. This was due to the formation of *in situ* depot from thermoresponsive system which allowed the improvement of control release pattern, indicating the inventiveness of our approach. However, before this approach can applied in the clinical treatment, several considerations are required. The sterility of this system should be considered. With regard to this, previous study has shown that the penetration of microorganism across the epidermis following the application of microneedles was found to be negligible [29]. Therefore, we could assume that the pores created by the use of microneedle should not cause the risk of infection. However, further studies are required to ensure this in the clinical applications. Moreover, the storage stability of the thermoresponsive gel should be investigated. It is also critical to ensure the reproducibility of this combination approach performed by patients. To achieve this, the pressure applied should be similar. Automated dermaroller has been developed to ensure the reproducibility of pressure applied [30], which could be potentially combined with the thermoresponsive developed in this study. Following this promising results, further *in vivo* studies should be carried out to investigate the efficacy, the pharmacokinetic profile and the dose determination of this approach. Moving forward, the impact of the deposition of the polymers should be evaluated. The use of Pluronics-based in the skin administration following microneedle applications is still limited. Therefore, the attention should not only be given to the pharmacokinetic profiles of the drugs, but also to the polymers used, particularly in the clearance profile. Finally, the scale-up manufacturing process should be considered in order to ensure that the method preparation can be applied in the industrial scale.

## 4. Conclusion

In this study, for the first time, we developed thermoresponsive hydrogels with bioadhesive properties to transdermally delivery PMQ combined with Dermarollers®. The combination of PF127 and PF68 with the ratio of 15% and 3% with 0.4% HPMC was found to be the optimal hydrogel with suitable thermoresponsive and mucoadhesive characteristics. The approach could potentially sustain the release of PMQ over 24 h. Essentially, the combination with Dermarollers® was able to enhance and control the *ex vivo* permeation and retention of PMQ through rats' skin over 72 h.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

## AUTHORS CONTRIBUTION

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript

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MINISTRY OF EDUCATION CULTURE OF INDONESIA
HASANUDDIN UNIVERSITY
**FACULTY OF PHARMACY**
Alamat Jalan Perintis kemerdekaan Km.10, Makassar 90245
Telepon (0411) 588556, Faksimili (0411) 590663
Laman: farmasi@unhas.ac.id

**The Editor**

**Colloids and Surfaces B: Biointerfaces**

August 9, 2022

Dear Sir/Madam,

I wish you to re-consider our manuscript entitled "Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®**"** for publication in **Colloids and Surfaces B: Biointerfaces.**

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 the response to the reviewer file. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript.

We believe that this article will be of great importance to drug delivery scientisct especially who are working on the use of polymers and biomaterials in several applications of drug delivery system. We declare that this article is original. The article has been written by the stated authors who are ALL aware of its content and approve its submission. This manuscript has not been previously published in any language anywhere and that it is not under simultaneous consideration by another journal. We have no conflict of interest exicts. If accepted, the article will not be published elsewhere in the same form, in any language, without the written concent of the publisher.

We appreciate your attention. We hope you will now consider publishing our research in **Colloids and Surfaces B: Biointerfaces** and look forward to hearing from you in due course.

**Yours Sincerely,**

**Andi Dian Permana (on behalf of all authors)**

**Faculty of Pharmacy**

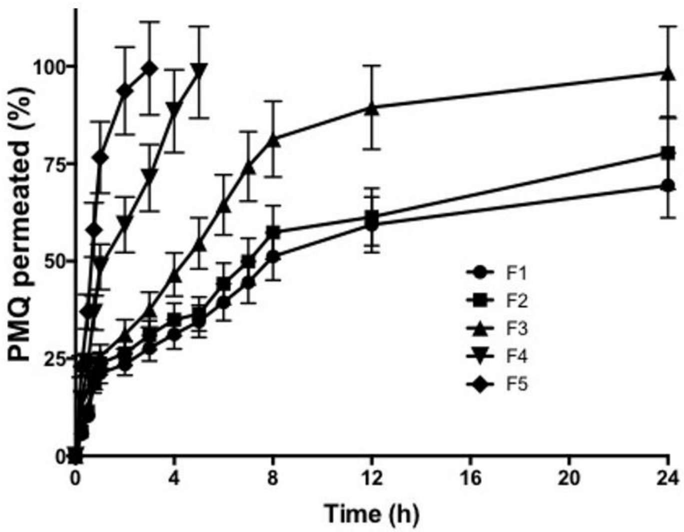
**Hasanuddin University**

**Indonesia**

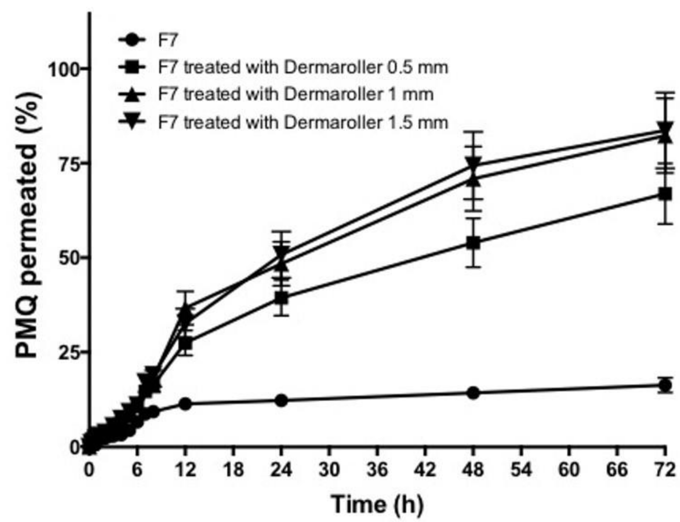
**Email: andi.dian.permana@farmasi.unhas.ac.id**

Graphical Abstract

Thermoresponsive hydrogel of primaquine




Controlled release over 24 h




Sustained skin permeation over 72 h

CRediT authorship contribution statement

**Andi Dian Permana:** Conceptualization, Methodology, Funding acquisition, Resources, Validation, Supervision, Writing – original draft. **Diany Elim:** Conceptualization, Data curation, Methodology, Funding acquisition, Resources. **Putri Wulandari Resky Ananda:** Methodology, Formal analysis, Investigation, Visualization. **Hilman Syamami Zaman:** Methodology, Investigation, Data curation. **Wahdaniyah Muslimin:** Data curation, Software, Validation. **Muhamad Gilang Ramadhan Tunggeng:** Methodology, Investigation

**Highlights:**

- Polymeric thermoresponsive hydrogels containing primaquine was prepared
- Hydrogels showed desired properties for thermoresponsive-mucoadhesive preparations
- *Ex vivo* transdermal delivery was enhanced when combined with Dermarollers®

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

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## Your Submission

1 message

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**Deborah Leckband** <em@editorialmanager.com> Tue, Aug 23, 2022 at 4:04 AM
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Ms. Ref. No.: COLSUB-D-22-01410R1
Title: Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®
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Dear Dr. Andi Dian Permana,

I am pleased to confirm that your paper "Enhanced and sustained transdermal delivery of primaquine from polymeric thermoresponsive hydrogels in combination with Dermarollers®" has been accepted for publication in Colloids and Surfaces B: Biointerfaces.

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