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Re: Combination of transdermal patch and solid microneedles for improved transdermal delivery of primaquine
Research Paper
Putri Wulandari Resky Ananda; Diany Elim; Hilman Syamami Zaman; Wahdaniyah Muslimin; Muhamad Gilang Ramadhan Tunggeng; Andi Dian Permana

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Your Submission IJPHARM-D-21-02344

1 message

International Journal of Pharmaceutics <em@editorialmanager.com> Wed, Sep 29, 2021 at 4:58 AM
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Comments on your paper have now been received and are attached to this message. Please consider all the points made and upon returning your paper detail your responses and the actions taken.

Please submit the revised manuscript by Nov 27, 2021. Upon receipt of your revised paper, we will inform you of the outcome as soon as possible. Papers not received within that that time period will be considered to be withdrawn, you are welcome to resubmit your paper as a new submission at a later date.

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Reviewers' comments:

Reviewer #1: The manuscript describes a transdermal drug delivery system designed for malaria treatment. I think that the system described here is simple and can provide advantages for the treatment of malaria. I think that the

article is well written and discussed. I have a few minor points that I think that the authors should address before the article can be accepted for publication:

1. The caption for Figure 4 does not provide information about what formulation were 1 and 2.
2. The discussion of FTIR and DSC can be misleading. When the presence of PMQ peaks in the FTIR of the patch does not mean that there are no interactions between the polymers and the drug. That confirms that the drug is present in the patch. Peak shifts is more indicative of non-covalent interactions. Moreover, if the drug is in amorphous state inside the patch, that suggest some sort of interactions between the polymers and the drug. I think that this should be presented in a more clear way to the reader.
3. I think that the mathematical modelling is not well implemented as all the correlation coefficients are quite low. The authors have biphasic release patterns: a zero order release in the first section of the profiles as the curves are mostly linear. And subsequently, a change in the slope (faster drug delivery). The authors achieved a patch capable of providing a nice zero order release over the first hours and that is really good as no burst release was observed initially. I suggest to apply a zero order model to the linear region only and then describe what could happen to the system to generate that change in the release rate (maybe water absorption?). I think that there is no point on using all these other models as they are not going to present a good fit to this particular release curves.

Reviewer #3: This manuscript designed a formulation that combined a transdermal patch containing primaquine (PMQ) with solid microneedles (Dermaroller ®). Microneedles were expected to help PMQ of the patch to diffuse through skin barriers by creating microchannels. The basic characteristics, permeation study, and irritation and histopathological evaluations in vivo of this system were evaluated. Overall, the whole research work presented a promising combination therapy. This valuable research work can be published after the authors provide more necessary data and revise the manuscript. Some suggestions are as follows.
1. It is recommended that Figure 1 should be added to the supplementary data.
2. In Figure 4 legend, please indicate what substances 1 and 2 represented.
3. Line 393, "Figure 5D" should be changed to "Figure 5D, E".
4. Given the focus of the combination of solid microneedles and transdermal patches, the advantages of this combination prescription than traditional solid microneedles (free drugs were applied directly after microneedles create microchannels) could not be shown. Relevant experiments need to be supplied.
5. There are lots of errors caused by carelessness in the manuscript. And English writing should be further improved. The authors should read over the manuscript carefully and correct all these mistakes. Some examples are as follows:
Line 59, "This study focuses on the treatment of malaria using PMQ …" should be in the past tense, and the sentence structure was chaotic.
Line 74-76, one clause and two main clauses appeared in one sentence.
Line 76-78, the sentence is hard to understand.
Line 99, "from" should be "form".
Line 108-109 and line 115, The full name of HPMC was wrong.
Line 160, "cm" should be "cm2".
Line 188, the unit of temperature is defective.
Line 265-266, the presentation of the literature citation was wrong.
Line 393, "Figure 5D" should be changed to "Figure 5D, E".
Line 400, "Figure x", unclear semantics.

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International Journal of Pharmaceutics

Combination of transdermal patch and solid microneedles for improved transdermal delivery of primaquine

--Manuscript Draft--

Manuscript Number:	IJPHARM-D-21-02344R1
Article Type:	Research Paper
Section/Category:	
Keywords:	Primaquine; Transdermal patch; Dermaroller®; transdermal delivery
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	Muhamad Gilang Ramadhan Tunggeng
	Andi Dian Permana, Ph.D
Abstract:	Malaria caused by various types of Plasmodium has become a global health problem. One of the drugs used as the first line of malaria therapy is primaquine (PMQ). PMQ isgenerallyadministered through theoral route. However, the use of PMQ orally could potentially cause some side effects and undergo the first-pass metabolism in the liver, reducing its effectiveness. Therefore, it is necessary to develop another drug administration route to avoid this effect. In this study, for the first time, PMQ was formulated into a transdermal patch for transdermal delivery, combined with solid microneedles, Dermaroller ® . Following several optimizations, HPMC and glycerin were used asthe main polymer and plasticizer, respectively. Specifically, the concentration of PEG 400 as apermeation enhancer was also optimized. The transdermal patches were evaluated for weight uniformity, thickness, surface pH, folding endurance, moisture content, moisture absorption ability, bioadhesive evaluation, and drug content recovery. PMQ release and permeation were also investigated through in vitro and ex vivo tests on rats' skin tissue. Importantly, the safety of the transdermal patch was also evaluated through in vitro hemolytic and in vivo irritation tests which were confirmed by histopathological examinations. The results showed that all formulations showed desired physical and bioadhesive properties with a folding endurance of>300 folds. The results exhibited that 31.31 ± 5.25% and 22.55 ± 4.35% of primaquine were released from transdermal patches following the in vitro and the ex vivo permeation studies. Combined with Dermaroller ® , the ex vivo permeation study showed an improved permeation profile with 45.89 ± 5.00% of primaquine permeated after 24 h with a zero-order kinetic during the first 8 h.Hemolysis percentage was found to be < 5%, indicating thenon-toxic of this approach. Finally, the histopathology studyshowed that there was no severe tissue damage following the administration of our approach. Further in vivo evaluations should be performed.

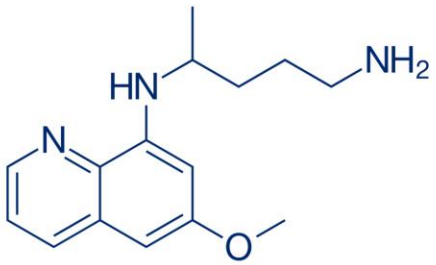
Declaration of interests

☒ 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:

Graphical Abstract (for review)

Combination of Transdermal Patch and Solid Microneedles for Improved Transdermal Delivery of Primaquine



Primaquine (PMQ)

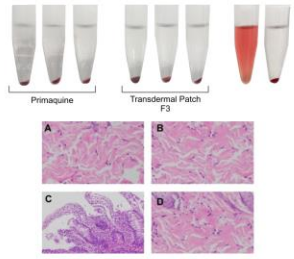
Transdermal Patch

- Uniformty of weight
- Folding Endurance
- Thickness
- Moisture Content
- Surface pH
- Bioadhesive Evaluation
- Moisture Absorption Ability
- Water Vapor Transmission
- DSC & FTIR
- Drug Content

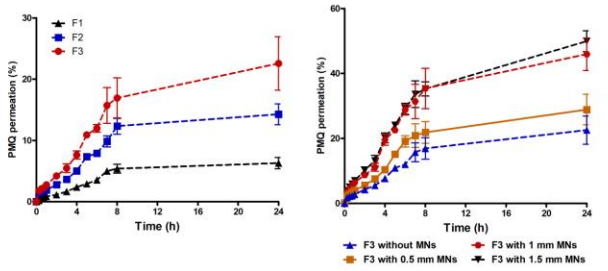
Characterization of transdermal patches

Solid microneedles

In vitro release study and *ex vivo* permeation study



Hemolytic activities and histopathological test



Enhance the penetration of PMQ through skin tissue with solid microneedles

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The Editor

International Journal of Pharmaceutis

October 1, 2021

Dear Abdul W Basit, PhD

I wish you to consider our manuscript for publication in **International Journal of Pharmaceutis** with the tiltle "**Combination of transdermal patch and solid microneedles for improved transdermal delivery of primaquine**"., We have addressed all comments from all reviewers as shown in "Response to Reviewer" file. Importantly, we have performed several additional experiments to address the suggestions from the reviewer. Also, we have re-checked the manuscript thoroughly and made significant changes to the grammatical and English errors.

The manuscript has not been previously published in any language anywhere and it is not under simultaneous consideration by another journal. We have no conflicts of interest.

We appreciate your attention. We hope you will now consider publishing our research in **International Journal of Pharmaceutis** 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

Credit Author Statement

Putri Wulandari Resky Ananda: Conceptualization, Methodology, Funding Acquisition, Writing - Original Draft, Review & Editing**; Diany Elim:** Conceptualization, Methodology, Funding Acquisition, Writing - Original Draft, Review & Editing**; Hilman Syamami Zaman:** Methodology, Writing - Original Draft**; Wahdaniyah Muslimin:** Methodology, Data Curation Writing - Original Draft**; Muhamad Gilang Ramadhan Tunggeng:** Data Curation, Validation, Writing - Original Draft**; Andi Dian Permana:** Conceptualization, Review & Editing, Project Administration, Funding Acquisition, Validation, Supervision**.**

Combination of transdermal patch and solid microneedles for improved transdermal delivery of primaquine

Putri Wulandari Resky Ananda[1], Diany Elim[1], Hilman Syamami Zaman, Wahdaniyah Muslimin, Muhamad Gilang Ramadhan Tunggeng, Andi Dian Permana*

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[1] **Equal Contribution**

Abstract

Malaria caused by various types of *Plasmodium* has become a global health problem. One of the drugs used as the first line of malaria therapy is primaquine (PMQ). PMQ is generally administered through the oral route. However, the use of PMQ orally could potentially cause some side effects and undergo the first-pass metabolism in the liver, reducing its effectiveness. Therefore, it is necessary to develop another drug administration route to avoid this effect. In this study, for the first time, PMQ was formulated into a transdermal patch for transdermal delivery, combined with solid microneedles, Dermaroller®. Following several optimizations, HPMC and glycerin were used as the main polymer and plasticizer, respectively. Specifically, the concentration of PEG 400 as a permeation enhancer was also optimized. The transdermal patches were evaluated for weight uniformity, thickness, surface pH, folding endurance, moisture content, moisture absorption ability, bioadhesive evaluation, and drug content recovery. PMQ release and permeation were also investigated through *in vitro* and *ex vivo* tests on rats' skin tissue. Importantly, the safety of the transdermal patch was also evaluated through *in vitro* hemolytic and *in vivo* irritation tests which were confirmed by histopathological examinations. The results showed that all formulations showed desired physical and bioadhesive properties with a folding endurance of >300 folds. The results exhibited that 31.31 ± 5.25% and 22.55 ± 4.35% of primaquine were released from transdermal patches following the *in vitro* and the *ex vivo* permeation studies. Combined with Dermaroller®, the *ex vivo* permeation study showed an improved permeation profile with 45.89 ± 5.00% of primaquine permeated after 24 h with a zero-order kinetic during the first 8 h. Hemolysis percentage was found to be < 5%, indicating the non-toxic of this approach. Finally, the histopathology study showed that there was no severe tissue damage following the administration of our approach. Further *in vivo* evaluations should be performed.

Key words: Primaquine, transdermal patch, Dermaroller®, transdermal delivery

1. Introduction

Malaria is a disease caused by parasites of the genus *Plasmodium*, a parasite belonging to the phylum Apicomplexa. The four species of plasmodium that commonly cause malaria in humans are *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, and *Plasmodium ovale* (Price et al., 2020). The type of mosquito that often infects humans is the female Anopheles. These mosquitoes mainly live in the tropics and subtropics regions. These mosquitoes are often found biting at dusk and dawn and with different amounts for each species (Price et al., 2020).

According to WHO data in 2019, an estimated 229 million cases of malaria occurred worldwide, an increase from 228 million cases in 2018. In 2019, about 51.7% of malaria cases caused by *P. vivax* occurred in Southeast Asia. Indonesia is the second country with the most malaria cases in Southeast Asia after India (WHO, 2020). In an effort to treat malaria, the therapy that can be given is to kill the malaria parasite in the body (WHO, 2015). Currently, antimalarial drugs are administered intravenously with injections and orally with tablet dosage forms. Some drugs that are still used and are not resistant to malaria parasites are doxycycline, mefloquine, quinine, and primaquine (PMQ) (WHO, 2016). In Indonesia, PMQ is the first-line drug used in malaria patients (PIONAS, 2015). A previous study focusing on the treatment of malaria using PMQ has shown that this drug was effective against all gametosids of all *plasmodium.* Therefore, this drug has been considered to be effective to break the chain of the spread of malaria (Nugraha, 2014).

However, when administered orally, several side effects have been reported, such as megaloblastic anemia, hematolytic, digestive disturbances, abdominal stiffness and epigastric pain. In addition, although pharmacokinetic studies of this drug have shown that PMQ has good absorption, it can be metabolized rapidly, undergoing first-pass metabolism in the liver, which can reduce the effectiveness of the drug. Moreover, PMQ has been reported to possess a short elimination half-life ($t_{1/2}e$). Therefore, the drug levels are quickly excreted in the blood (Mayorga et al., 1996).

According to the issues mentioned above, it is necessary to develop a drug delivery system that can solve the problems. The transdermal delivery system was considered to be an appropriate approach because this system could potentially provide uniform plasma concentrations, avoid the symptoms of first-pass metabolism, and control the dose frequency. With all these benefits, the drug could potentially be delivered to the desired target and suppress side effects (Zhang et al.,

2014). When the drug is given through this route, it will prevent the degradation of the drug in the liver or in the digestive tract. Some drug molecules undergoing extensive first-pass effect have been formulated into transdermal dosage forms, including testosterone, nitroglycerin and methyl salicylate (Pastore et al., 2015). With respect to the dosage form of transdermal delivery, transdermal patches have been considered as one of the most effective forms. Transdermal patches have solid dimensions and uniform thickness (El-Gendy et al., 2009). Accordingly, the administration of this system could ensure the uniformity of the dose applied. Essentially, unlike other topical dosage forms, transdermal patches would stay in the skin during the application time without being removed (Rahman et al., 2021). Therefore, these benefits could provide a sustained and controlled release of drugs incorporated into this delivery system.

To enhance the permeation of the drugs through the skin, solid microneedle (SMN) can be used as a combination with transdermal patch dosage forms. This SMN would be able to help the drug from the formulation to diffused through *stratum corneum* in the skin barriers by creating microchannels (Ita, 2015). The SMNs that are commonly used (0.25 mm to 2.0 mm) penetrate to the dermis of the skin and would enhance the permeation of a drug formulation through a slow diffusion from the pores into systemic capillaries (Kim et al., 2012; Waghule et al., 2019). With all these advantages, it was hypothesized that SMN would be an excellent match with the transdermal patch to further improve the penetration of PMQ *via* the transdermal route, reaching the system circulation. Furthermore, different from other needles, SMN does not cause a painful outcome because it does not reach the pain receptors yet. Thus, it would be painless and improve patient compliance when using it (Waghule et al., 2019). In addition, SMN is safe due to their small size and length, also possible to be used self-administered and reduce the need for expertise to apply the dosage forms (Jung and Jin, 2021). For the first time, in this study, we formulated a polymeric patch containing PMQ for transdermal delivery using hydroxypropyl methyl cellulose (HPMC) as the main polymeric matrix. The transdermal patches were characterized and evaluated for their physical property, bioadhesive property, moisture absorption ability, water vapor transmission, FTIR and DSC studies, as well as *in vitro* permeation profiles. Essentially, the *ex vivo* permeation profiles in full-thickness rat skin were also investigated with the combination with SMN for enhanced transdermal delivery. In this study, Dermaroller® was used as a type of SMN. Dermaroller® would pierce the stratum corneum, creating holes in the skin and, therefore, enhance the penetrability of PMQ from the transdermal patches. Several studies have shown the

effectiveness of Dermaroller® to improve the transdermal delivery of drugs (Ahad et al., 2017a, 2017b; Badran et al., 2009). Finally, the *in vitro* hemolytic test and the *in vivo* skin irritation observed by histopathological evaluation were finally assessed to investigate the possible toxicity and irritation effects of this approach.

2. Materials and method

2.1 Materials

Primaquine biphosphate (purity of 98%), hydroxypropyl methyl cellulose (HPMC) (Poly(ethylene glycol) (PEG) 400, polyvinyl pyrrolidone (PVP), glycerin, anhydrous calcium chloride, magnesium chloride, sodium nitrite and potassium sulphate were obtained from Sigma-Aldrich Pte Ltd. (Singapore, Singapore). Solid microneedles (Dermarollers®) were purchased from SQY® (Guangdong, China). Other chemicals were analytical grade.

2.2 Formulation of transdermal patches

Transdermal patches containing PMQ were prepared using several types of polymers. In this study, HPMC and PVP were screened as the polymers. Initially, the polymers were dissolved in water in a glass beaker until homogeneous using an Ultra-Turrax® homogenizer (IKA, model T25, impeller 10 G, Germany). After homogenization, PMQ, glycerin and polyethylene glycol-400 (PEG-400) were added and stirred again using an Ultra-Turrax® homogenizer until homogeneous. To reduce bubbles due to stirring, the formula was placed into a centrifuge tube and spun for 15 minutes at 3000 rpm (LC-04S Centrifuge, Zenith Lab (Jiangsu) Co., LTD.). The formulation was poured as much as 10 grams on the petri dishes and then dried in a box containing silica (Sheth and Mistry, 2011).

2.3 Evaluation of transdermal patches

2.3.1 Physical appearance

Visual appearance, including the color, clarity, flexibility and smoothness of all prepared patches were examined.

2.3.2 Thickness of the patch

Patch thickness was measured using Vernier calipers on several parts of the patch. Patch thickness measurement was done by clamping the patch on the measuring tool at different parts of the patch. The measurement results obtained were averaged, and the standard deviation value was calculated for each patch.

2.3.3 Uniformity of weight

The patches were tested for weight variations by measuring the weight of all patches. Three patches measurement was performed for each formulation. The average of the patch weight and standard deviation were determined.

2.3.4 Folding endurance

This study was carried out to evaluate the strength of the patches. Folding endurance was assessed by the number of folds on the patch in the same place until the patch breaks or cracks. The number of folds was expressed as the value of folding endurance (Singh and Bali, 2016).

2.3.5 Drug content and content uniformity determination

Measurement of drug content was carried out by dissolving the patch into 10 ml of phosphate buffer (pH 7.4) until homogeneous. The drug content was analyzed using a UV-Vis spectrophotometer at 262 nm (Ali and Hanafy, 2016). The content uniformity of PMQ in the patch formulations was also determined. Briefly, five parts from different are of the patch were taken. The PMQ concentration was then measured using the method used to determine drug content.

2.3.6 Surface pH

In this study, the patch was weighed as much as 20 mg, then stored in a beaker containing 50 ml of double distilled water. Furthermore, they were allowed to swell for 15 minutes at room temperature. A combined glass electrode was located near the surface of the patch to be measured and pH measurements were conducted after equilibration time of 1 min.

2.3.7 *In vitro* bioadhesive evaluation

The bioadhesive strength of the transdermal patches was assessed using an adapted physical balance with a slight modification (Rahman et al., 2021). Initially, 2 vials were placed on one side of a physical balance where the upper vial was given weight. The bottom of the vial was positioned upside down and rat skin was attached. Afterwards, patches were applied to the skin. On the other side, 1 gram of weight was added every 30 seconds until the vial was separated. This method was carried out at 37°C. The bioadhesive strength was determined using the following equation (1).

$$\text{Bioadhesive strength } (N/m^2) = \frac{\text{mass x 981}}{\text{1000 x Skin surface are}} \quad (1)$$

2.3.8 Moisture absorption ability (MAA)

The patches were cut in sizes of 1x1 cm^2 and were placed in three desiccators containing magnesium chloride (33% RH), sodium nitrite (65% RH) and potassium sulfate (97% RH)

(Rahman et al., 2021). The patches were weighed every 24 hours for 7 days. The percentage of moisture absorption ability was calculated by the following equation (2).

$$\%MAA = \frac{\text{final mass of patch - initial mass of patch}}{\text{initial mass of patch}} \times 100\% \quad (2)$$

2.3.9 Water vapor transmission (WVT)

Initially, 1 g of anhydrous calcium chloride was put into a dry glass vial. The transdermal patch was secured to seal the vial using an adhesive tape. The vials were located into a desiccator containing potassium chloride (saturated solution). At the predetermined time, the glass vial was taken and accurately weighed. Finally, water vapor transmission was calculated using the following equation (Basha et al., 2011):

$$WVT = \frac{\text{mass of vial x thickness of patch}}{\text{surface area of patch}} \quad (3)$$

2.4 Fourier Transform Infrared (FTIR) spectroscopy

FTIR spectrophotometry was used to evaluate the compatibility of PMQ with all excipients used in the transdermal patch preparation. The sample used was analyzed at a wavelength of 400 to 4000 cm^{-1} using an FTIR Accutrac FT/IR-4100 Series (Jasco, Essex, UK) (Singh and Bali, 2016).

2.5 Differential scanning calorimetry (DSC)

Thermal analysis by DSC was carried out to investigate the physical state of PMQ in the patch formulation. The analysis was performed using the DSC model Q20 V24.2 Build 107 (Universal V4.5A TA Instruments). The formulations were put into an aluminum pan and then heated at a temperature of 25-300°C at an increased speed of 5°C/min (Singh and Bali, 2016).

2.6 *In vitro* permeation study

In vitro permeation study was performed using Franz diffusion cells with dialysis membrane between donor and receiver compartment. PBS pH 7.4 was used as release media. The experiment was carried out at 37 ± 0.5°C and stirred at 100 rpm. Sampling was carried out at time intervals of 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours by taking 1 ml of receiver compartment and replacing it with PBS after sampling. The samples were then analyzed using UV-vis spectrophotometry at 262 nm.

2.7 *Ex vivo* permeation and retention studies

Ex vivo permeation study was conducted using a method similar to *in vitro* permeation study. Instead, abdomen part of Wistar rats' skin was used in this study (Permana et al., 2019a, 2019b, 2020a). Additionally, the effect of the combination of the transdermal patch with Dermaroller®

was investigated. Briefly, prior to the application of the patch, Dermaroller® was manually applied to the skin. Three different needle lengths of Dermaroller® was used in this study, namely 0.5 mm, 1 mm and 1.5 mm. In this study, the permeation of PMQ from the solution was also evaluated. In this study, the concentration of PMQ deposited in the skin tissue was also investigated. Briefly, the skin samples after 1 h, 2 h, 4 h, 8 h and 24 h of the administration of transdermal patches and solution, combined with Dermaroller® were removed from Franz diffusion cells. The skin tissues were washed with PBS pH 7.4 to remove the excess formulations in the surface of the skins. The skin tissues were mixed with 20 mL methanol and homogenized using an Ultra-Turrax® homogenizer for 15 min. The mixture was then centrifuged for 30 min at 5000 rpm. The concentrations of PMQ in the supernatant were then quantified using UV-vis spectrophotometry at 262 nm.

2.8 Hemolytic test

Initially, the erythrocytes of Wistar rats were separated from the blood by centrifugation for 20 min at 2000 rpm. The erythrocytes obtained were washed using PBS for three washing cycles. After that, the erythrocytes were resuspended in PBS to obtain a final suspension at 10% v/v concentration. After obtaining a cell suspension of 100 µL volume, the suspension was added to 900 µL of the sample containing PMQ with a concentration of 500 µg/mL, 50 µg/mL and 5 µg/mL. For the following process, the mixture was incubated at 37°C for 60 minutes, followed by centrifugation at 7000 rpm for 10 minutes. Lastly, the results of supernatant centrifugation were measured using UV-Visible spectroscopy at 540 nm to estimate free hemoglobin. As positive and negative hemolytic controls, PBS and distilled water were used, respectively. After all the steps were carried out, the serum and plasma color changes were observed to analyze the hemolysis of the sample (Mir et al., 2020). Each experiment carried out in this method was carried out in triplicate for each concentration. Hemolysis percentage was determined using equation 4:

$$\text{Hemolysis (\%)} = \frac{\text{Absorbance (Test sample) - Absorbance (Negative control) x 100}}{\text{Absorbance (positive control) - Absorbance (Negative control)}} \quad (4)$$

2.9 *In vivo* skin irritation and histopathological evaluations

The *in vivo* irritation test was carried out in Wistar rats (n=4). The study was permitted by the Ethical Committee of the Faculty of Medicine, Hasanuddin University, Indonesia. Initially, the hair of the rats' back was shaved. Following that, several treatments were performed, namely patch application, Dermaroller® and patch applications, sodium lauryl sulphate as positive control and

untreated groups as a negative control. After 24 h, the patches were removed, and the animals were culled. The skin specimens were carefully taken for histopathological examination. All isolated specimens were kept in formaldehyde solution (30% v/v), and skin biopsies were performed. Afterwards, the specimens were stained using hematoxylin-eosin staining agents to envisage cells and their cytoplasmic portions of cells or tissues (Hussain et al., 2020).

2.10 Statistical Analysis

IBM® SPSS® Statistics 26.0 (IBM, Armonk, New York, USA) was utilized to statistically analyze all the data, p values < 0.05 were considered as significant differences.

3. Results and discussion

3.1 Formulation of transdermal patches

This study was carried out to transdermally deliver PMQ as an alternative delivery route of oral administration. In the preliminary study, we compared the use of PVP and HPMC as the polymeric matrix of the transdermal patches (data not shown). The results showed only HPMC resulted in patch preparations with desired characteristics. Patches prepared with PVP were too brittle and difficult to remove from the petri dishes. Furthermore, to achieve high drug loading in the patch preparation, the concentration of PMQ was optimized. The results showed that above 2% w/w, the prepared patches were found to possess poor mechanical properties. Accordingly, 2% w/w was selected as the concentration of PMQ in the patch formulation. Glycerin was used as a plasticizer. PEG 400 has been reported to act as a permeation enhancer in the transdermal preparation. In this study, we compared three different concentrations of PEG, as shown in Table. 1. It was important to note that above 1.5% w/w of PEG 400, the patches were too sticky and did not form dry and elastic patches. Accordingly, only three formulations were selected for further studies. Figure S1A, S1B and S1C show the representative images of F1, F2 and F3, respectively.

Table 1. Formulation of the transdermal patches of PMQ

Formula	Compositions (%w/w)				
	PMQ	HPMC	Glycerin	PEG 400	Aquadest
F1	2	2	0.5	1	ad 100
F2	2	2	0.5	0.75	ad 100
F3	2	2	0.5	1.5	ad 100

3.2 Evaluation of transdermal patches

3.2.1 Physical characterization

The initial characterization of the patch was the physical examinations. Table 2 depicts the uniformity weight, the uniformity thickness and folding endurance of the prepared patches. It was obviously observed that the RSD values of the weight and thickness were less than 5%. Therefore, it was concluded that all patches possessed uniform weight and thickness (Ali and Hanafy, 2016), allowing the uniform dose of PMQ in all parts of the patches. Furthermore, the folding endurance of the patch was assessed. It was carried out to examine the resistance ability and mechanical properties of the patches. As shown in Table 2, all formulations exhibited folding endurance values of > 300 times. Figures S1D, S1E and S1F show the images of folding endurance characterization of patches. It was postulated that a desired folding endurance value of the patch should be more than 300 times (Rahman et al., 2021). Accordingly, the patches prepared in our study showed adequate mechanical properties.

Table 2. Physical characterization data of prepared transdermal patches (Means ± SD, n= 3)

Formulation Code	Uniformity of Weight		Thickness		Folding Endurance
	Average ± SD	%RSD	Average ± SD	%RSD	
F1	0.83 ± 0.0047	0.5656	0.074 ± 0.0049	6.6202	> 300
F2	0.57 ± 0.0124	2.2009	0.084 ± 0.0049	5.8321	> 300
F3	0.65 ± 0.0081	1.2561	0.080 ± 0.0063	7.9056	> 300

3.2.2 Drug content determination

It was important to ensure that the concentration of PMQ was not affected in the patch formulation. Figure 1A depicts the results of the percentage recovery in three different formulations. The results show that the three formulas have recovery percentages of 98.17 ± 0.42%, 98.87 ± 1.49%, and 102.808 ± 0.34% for F1, F2 and F3, respectively. ICH has reported that a good percentage recovery is 95-105% (BioPharm International, 2007). Accordingly, this shows that the excipients and the method used did not affect the concentration of the PMQ in the formulations. Additionally, the concentration of PMQ distributed in all area of the patches was also determined. As shown in Table 3, following the determination of PMQ content in five parts of the patch, PMQ showed the acceptable recovery values, showing the homogeneity of PMQ in all part of the patches.

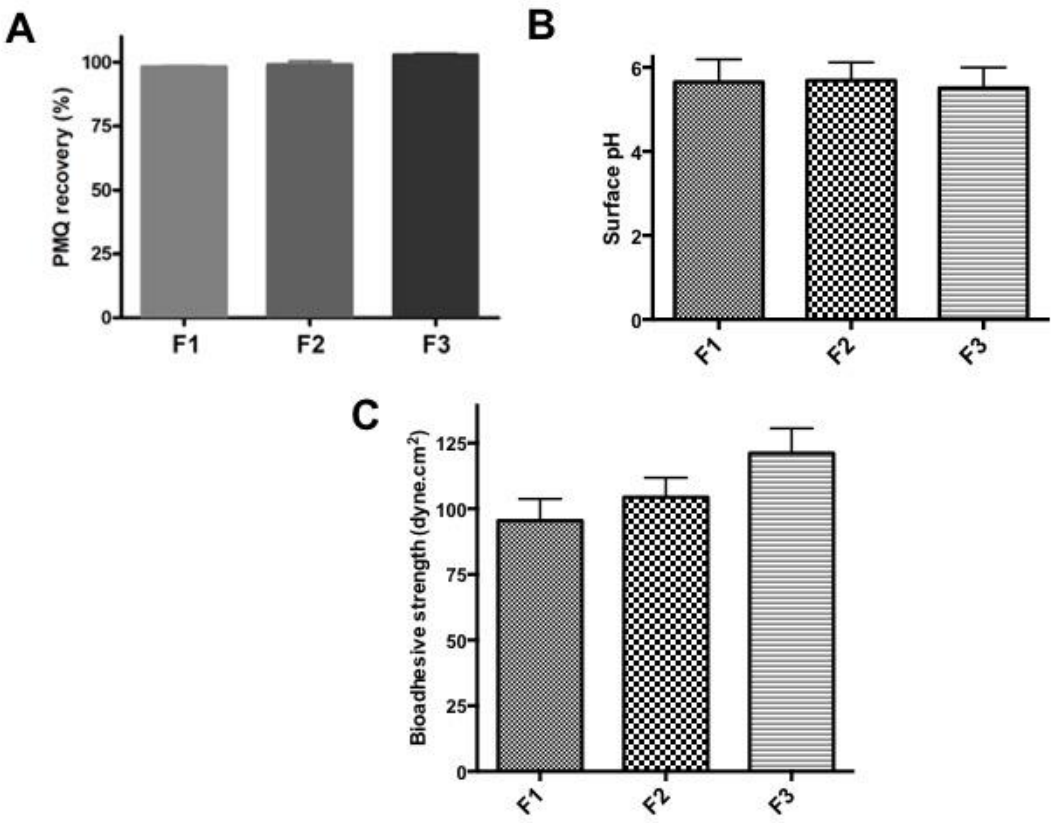

Figure 1. Drug recovery percentage of transdermal patches (mean ± SD, n= 3) (A); Surface pH values of transdermal patches (mean ± SD, n= 3) (B); Bioadhesive strength of transdermal patches (Means ± SD, n= 3) (C)

Table 3. Uniformity of content prepared transdermal patches (Means ± SD, n= 3)

Formulation Code	PMQ recovery (%)				
	Area 1	Area 2	Area 3	Area 4	Area 5
F1	98.45 ± 0.32	99.17 ± 0.29	98.39 ± 1.82	99.09 ± 0.35	98.42 ± 1.22
F2	99.31 ± 0.12	98.98 ± 1.23	99.37 ± 0.54	98.32 ± 1.34	99.09 ± 0.34
F3	99.78 ± 0.42	100.21 ± 0.32	98.98 ± 0.34	99.44 ± 0.54	101.23 ± 0.54

3.2.3 Surface pH

It was crucial to ensure that the surface pH of the topically applied dosage form could be tolerated by the skin. The unsuitable pH values could potentially irritate the skin, especially for sustained release formulation, which was applied to the skin for a long period. Accordingly, it is critical to evaluate the surface pH of the transdermal patches prepared. Figure 1B shows that the surface pH values of F1, F2 and F3 were 5.65 ± 0.51, 5.49 ± 0.44 and 5.68 ± 0.34, respectively. These values were relatively close to the skin pH, which is approximately 5.8 (Miksusanti et al., 2020). Thus, it

could be concluded that the administration of these patch would not irritate the skin and could be applied for long period to the skin.

2.2.4 *In Vitro* bioadhesive evaluation

As a topical preparation, it is important that the patch should be able to stay for a longer period in the skin. This would enable the improvement of the diffusion rate of the drug, providing high bioavailability (Rahman et al., 2021). Following the application to the skin, the polymer would be hydrated, forming an interaction with the mucous membrane in the skin. It has been previously reported that HPMC possesses mucoadhesive properties, making it ideal for bioadhesive preparation (Permana et al., 2021b, 2021a). Figure 1C shows the bioadhesive strength of all formulations. The results showed that the bioadhesive strength values of F1, F2 and F3 were found to be 95.43 ± 8.32 N/m^2, 104.34 ± 7.43 N/m^2 and 121.19 ± 9.43 N/m^2, respectively. It was observed that the increase of PEG concentration could improve the bioadhesive property of the patch. It has been previously reported that PEG also showed bioadhesive capability due to their interaction with mucin (Roy et al., 2009).

3.2.5 Moisture absorption ability

One of the crucial characteristics of a transdermal patch is the ability to absorb moisture from the environment. This property would affect the mechanical strength and release profile of the drugs incorporated into the patches. Here, three types of RH values were used. As depicted in Figure 2, there were increases in moisture absorption following the increase in RH values. Following 14 days, all formulations showed moisture absorption of less than 10%. It might be caused by the elastic structure of HPMC (Michailova et al., 2000). Therefore, this resulted in the slow moisture absorption and water uptake of the prepared patch. However, it should be noted that a small percentage of moisture content is still required to maintain the elasticity of the patch (Rasool et al., 2021).

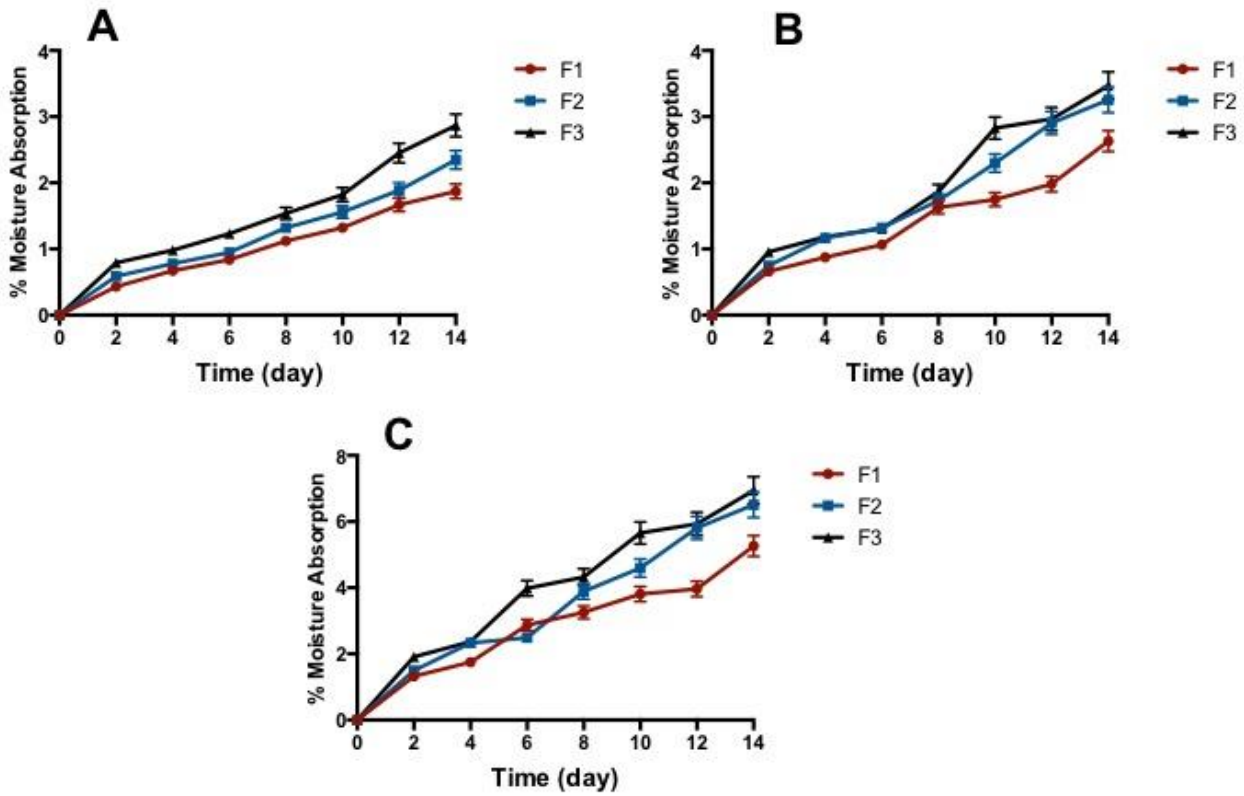

Figure 2. Percentage of moisture absorption of transdermal patches containing PMQ at 33% RH (A), 65% RH (B) and 97% RH (C) (Means ± SD, n= 3).

3.2.5 Water vapor transmission

Another stability parameter of patch is water vapor transmission ability. After 7 days, the rate values were calculated to be 0.45 ± 0.02 µg.cm/ cm^2 24 h, 0.49 ± 0.03 µg.cm/ cm^2 24 h and 0.54 ± 0.05 µg.cm/ cm^2 24 h for F1, F2 and F3. These values were considered to be lower compared to a reported study (Singh and Bali, 2016). Low WVT values could indicate the long-term stability of the patch.

3.3 Fourier Transform Infrared spectroscopy

FTIR spectroscopy was utilized to examine the interaction between PMQ and excipients. The spectra of pure PMQ and transdermal patch containing PMQ are depicted in Figure 3A. In PMQ spectrum, several peaks were observed at 3318 cm^{-1} for N-H stretch, 3012 cm^{-1} for C-H stretch, 1629 cm^{-1} for C=C stretch and 9954 cm^{-1} for C-O stretch. Importantly, all these peaks were also found in the transdermal patch, suggesting the presence of PMQ in the patch formulation.

However, it was interesting to note that the peak at 3318 cm^{-1} became broader, which could be due to the formation of hydrogen bonding (Permana et al., 2020b) between PMQ and the polymeric matrix, reducing the crystallinity of PMQ.

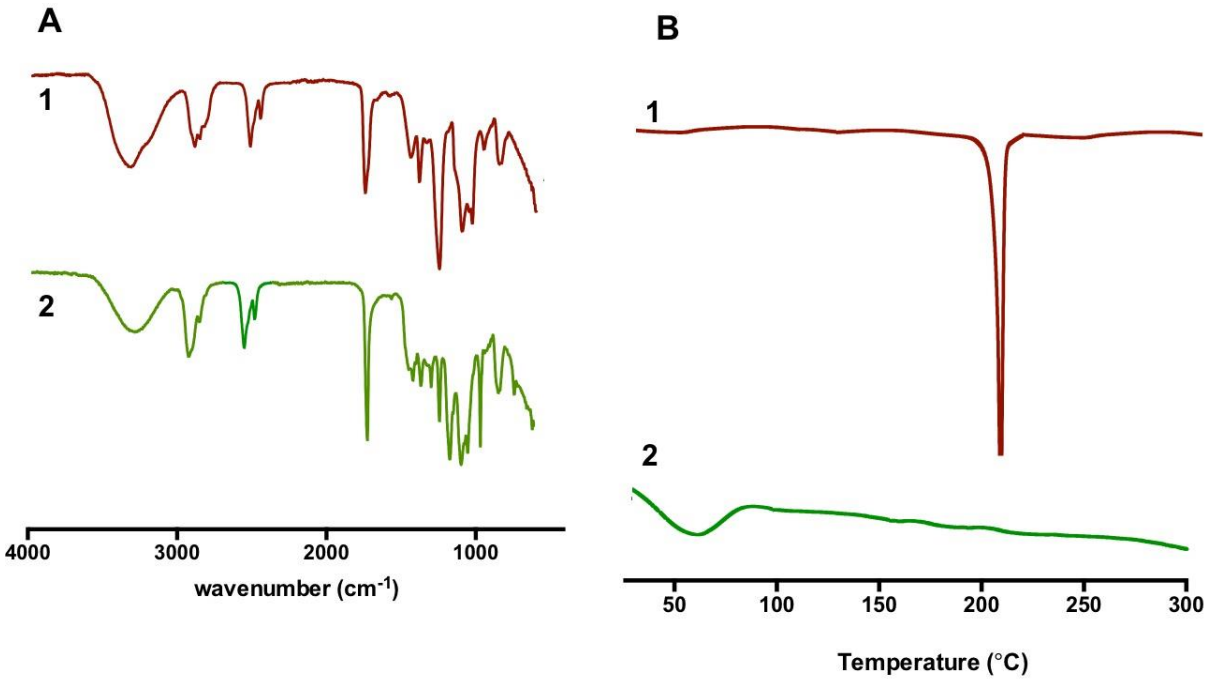

Figure 3. FTIR spectrum (A) and DSC thermogram (B) of PMQ (1) and transdermal patches containing PMQ (2).

3.4 Differential scanning calorimetry

DSC evaluation was carried out for PMQ and the transdermal patch containing PMQ. Figure 3B shows the result of this study. The result showed that there was an endothermic peak at 202.5°C, indicating the melting point and the crystallinity of pure PMQ. However, this peak was not found in the thermogram of transdermal patch, indicating that the PMQ changed to the amorphous state and was uniformly dispersed in the matrix of the polymeric patch. Additionally, we did not observe additional peaks in the transdermal patch thermogram. The absence of PMQ peak in the transdermal patch is consistent with the results from FTIR evaluation, showing that the possible presence of the hydrogen bonding could reduce the crystallinity of PMQ and form an amorphous state. A previous study has demonstrated similar phenomenon in the formulation of dipyridamole in vascular graft formulations (Domínguez-Robles et al., 2021).

3.5 *In vitro* permeation study

To evaluate the permeability of PMQ from the transdermal patch, *in vitro* permeation study was carried out. This evaluation was carried out to select the formulation for further studies. The selection was performed based on the highest permeation of PMQ detected in the receiver compartment. Figure 4A shows the *in vitro* permeation profiles of PMQ from three different formulations. The results obtained showed that after 24 hours, the formula F1 (PEG 0.75%), F2 (PEG 1%) and F3 (PEG 1.5%) showed the cumulative percentage values of 16.15 ± 0.39%, 20.76 ± 2.27% and 31.31 ± 5.25%, respectively. When analyzed statistically, there was a significant difference ($p < 0.05$) between these values. It was previously reported that PEG 400 could enhance permeation ability of several drugs in the transdermal preparations (Bolla et al., 2020; Singh and Bali, 2016; Zhang et al., 2020).

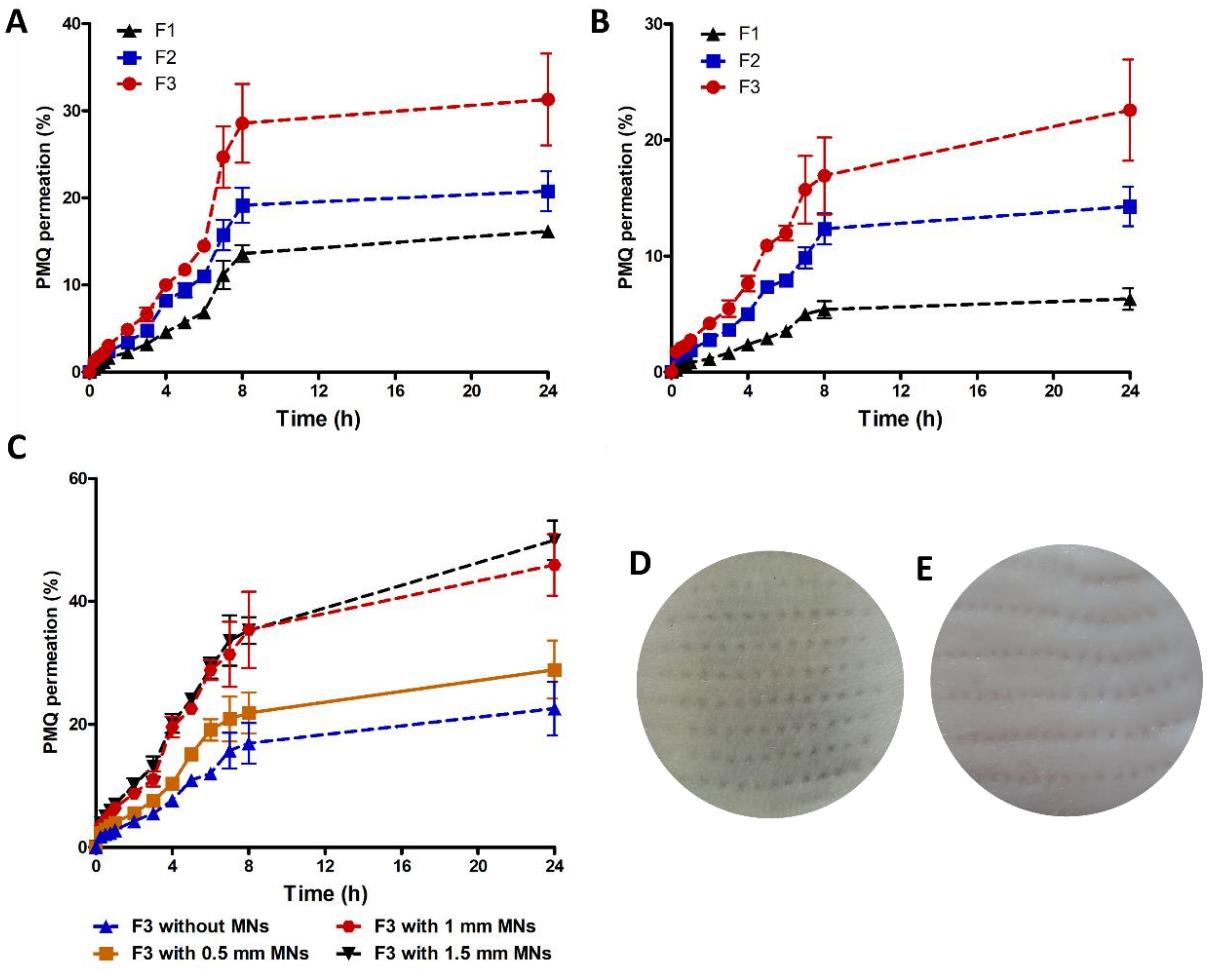

Figure 4. *In vitro* permeation study of the transdermal patches containing PMQ (mean ± SD, n= 3) (A); *Ex vivo* permeation study of the transdermal patches containing PMQ (mean ± SD, n= 3) (B); *Ex vivo* permeation study of F3 combined with Dermaroller® (mean ± SD, n= 3) (C); Representative images of the micropores on the skin created upon insertion of Dermaroller® of 0.5 mm (D) and Dermaroller® of 1 mm (E)

3.6 *Ex vivo* permeation and retention studies

Furthermore, the selected formulation was evaluated for its *ex vivo* permeation ability through rats' skin. In this evaluation, the highest permeation percentage of the three formulas was found in F3 with a value of 22.55 ± 4.35% after 24 hours, as shown in Figure 4B. This value was significantly higher ($p < 0.05$) than F1 (6.29 ± 0.92%) and F2 (14.25 ± 1.69%). Also, the results of the *ex vivo* test were similar to the results of the *in vitro* test, showing the permeation profile of the Higuchi model.

Based on the results above, F3, which had a better permeation percentage was continued in the *ex vivo* test combined with Dermaroller®. Various length needles were evaluated, namely 0.5 mm, 1 mm, and 1.5 mm lengths. The permeation profile of F3 combined with three different lengths of microneedles is shown in Figure 5C. Overall, the results of the three microneedle lengths were significantly different ($p < 0.05$) when compared to the permeation percentage of the initial formula. The lowest percentage of permeation using a combination of the transdermal patch and SMNs was 0.5 mm Dermaroller® with a value of 28.87 ± 4.70%. The test results with 1 mm SMNs and 1.5 mm SMNs were not significantly different ($p < 0.05$) with values of 45.89 ± 5.00% and 49.93 ± 3.18%, respectively. It was clearly observed that the use of Dermaroller® could improve the permeation of PMQ. It was due to the creation of a micropore in the skin, allowing the drug to permeate through the pore, enhancing the permeability of PMQ. As shown in Figure 4, the formulation of PMQ in transdermal patches could produce a sustained release manner of PMQ over 24 h. It has been previously reported that the uniform distribution of the drug in the patch formulations could also assure the uniform reproducible sustained release of the drug molecules from the patch (Arora and Mukherjee, 2002). Accordingly, the sustained release effect obtained in this study could also be due to the excellent uniformity content of PMQ in the patches. The pores created in the skin after the administration of Dermaroller® are shown in Figure 4D, E. Based on these results and the consideration of the convenience of using painless SMNs (Jung and Jin, 2021),1 mm Dermaroller® was chosen as the most effective combination with the transdermal patch of PMQ. It is important to note that a burst release was not observed in the *in vitro* and *ex vivo* evaluations. This might be due to the slow moisture absorption ability of HPMC, as shown in the determination of moisture absorption ability results, leading to a sustained and slow diffusion rate of PMQ from the patch formulation. After 8 hours, the permeation profile of F3 combined with 1 mm Dermaroller® showed a zero-order kinetic model with acceptable linearity (R2=

0.9786). Therefore, it could be a favour in transdermal delivery to provide a stable plasma concentration during the application of this patch preparation (Ma et al., 2021). It was also observed that the permeation profile exhibited a biphasic manner after a zero-order kinetic from 8 h to 24 h. During the first 8 h, PMQ in the surface might permeate first due to the water and moisture absorption. Afterwards, the PMQ permeated in a slower rate, providing a sustained release profile. The permeation of PMQ from the solution after the administration of Dermaroller® was further evaluated as a comparison to prove the effectiveness of our approach. As shown in Figure 5A, approximately 100% of PMQ permeated the skin only after 3 hours. Accordingly, it was proven that our approach could sustain the release of PMQ. Importantly, the concentrations of PMQ deposited in the skin following the administration of PMQ patch and solution, in combination with Dermaroller® with a length of 1 mm were determined. Figure 5B depicts the result of this experiment. As depicted, in solution, PMQ was only deposited after 2 h of administration, with only 165.21 ± 98.11 mg/g skin tissue localized in the skin. On the other hand, following the administration of transdermal patches, the concentration of PMQ deposited in the skin increased over 24 h, resulting in 687.65 ± 118.32 mg/g skin tissue localized in the skin. Accordingly, our results suggested that the administration of PMQ in transdermal patches, combined with Dermaroller®, could improve the permeation profile of PMQ, sustain the release of PMQ, and importantly, enhance the localization of PMQ in the skin over 24 h.

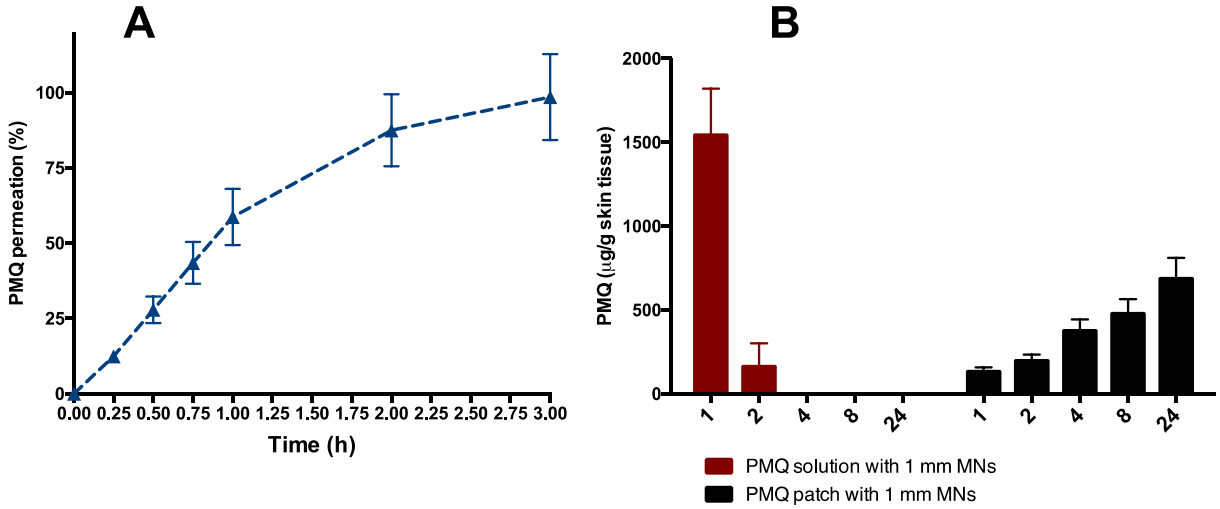

Figure 5. *Ex vivo* permeation study of PMQ from solution combined with Dermaroller® with a length of 1 mm (mean ± SD, n= 3) (A); *Ex vivo* retention study of PMQ from solution and transdermal patch combined with Dermaroller® with a length of 1 mm (mean ± SD, n= 3)

3.7 Hemolytic test

To evaluate the possibility of PMQ in the transdermal patch to show toxicity effect, a hemolytic assay was performed. This assay was useful to initially assess the toxicity of the new formulation. Figure 6 shows the result of this study. It was shown that PMQ solution and PMQ in transdermal patch did not show any toxicity, indicated by clear supernatant obtained after the incubation with red blood cells (Figure 6). Importantly, the percentage of hemolysis values were similar to the negative control, which were below 5%. The hemolysis index is considered safe when <5% (Zhou et al., 2011). Therefore, the results indicate that PMQ and transdermal patches could be considered to be safe when administered transdermally.

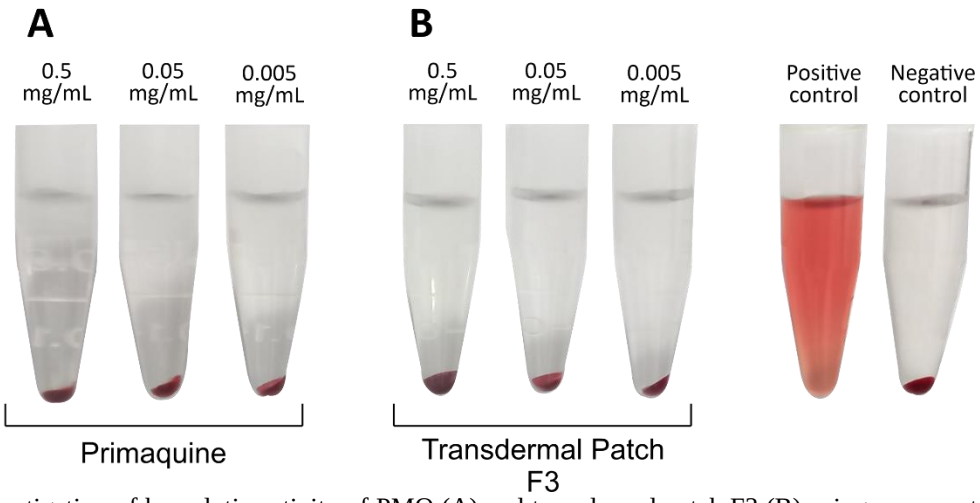

Figure 6. Investigation of hemolytic activity of PMQ (A) and transdermal patch F3 (B) using a concentration range from 0.005 mg/mL to 0.5 mg/mL, by comparing the color of samples with the controls.

3.8 *In vivo* irritation and histopathological evaluations

As controlled release formulation, applied in the skin for a long-trm period, it was crucial to evaluate the potential irritation caused by the application on the transdermal patch containing PMQ. To perform this study, histopathological evaluation using hematoxylin and eosin staining was carried out following the application of the patch for 24 h. Figure 7 shows the representative images of the skin area applied with the transdermal patch. As a positive control, a gel containing sodium lauryl sulphate as an irritant agent was used. Furthermore, untreated rats were used as a negative control. As shown, there was no indication of toxicity and irritation following the administration of the transdermal patch, as well as the combination of Dermaroller® and transdermal patch, indicating the safety of this approach. No irritation was also observed in the

untreated group. On the other hand, in the positive control group, skin damage was observed, with infiltration and erosion were observed in histopathological results. Accordingly, it was concluded that the combination of Dermaroller® and the transdermal patch was considered to be safe as it did not result in any irritation and tissue damage.

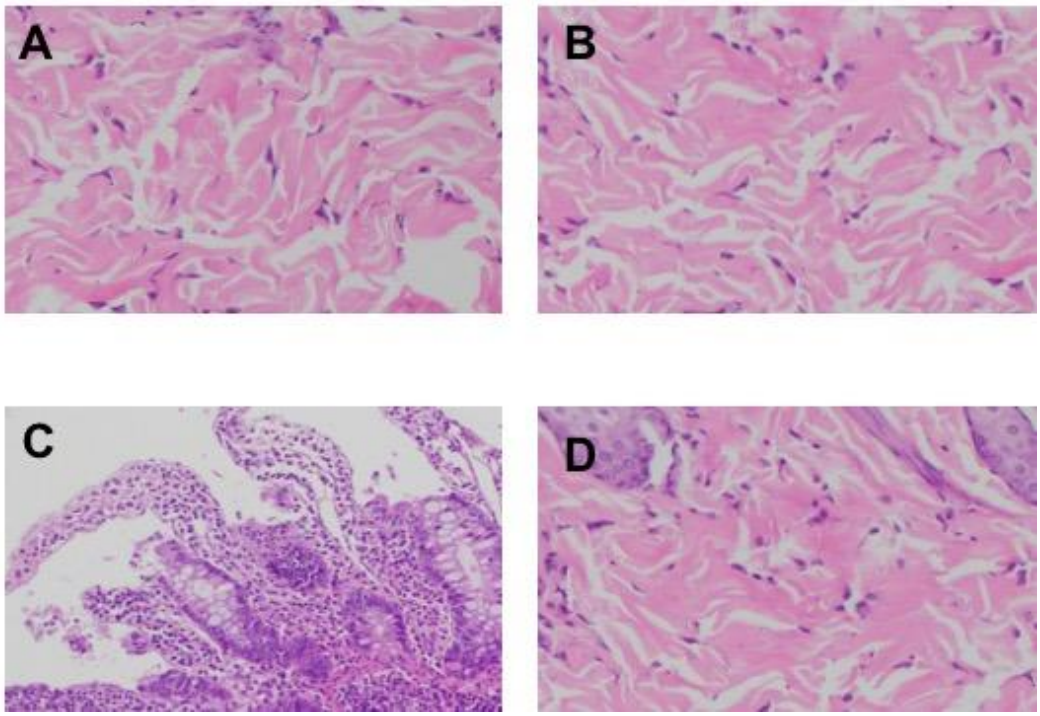

Figure 7. Histopathological test skin rat with patch (A); Histopathological test skin rat with Microneedle and patch (B); Positive control (C); Negative control (D).

The overall results obtained in this study showed the promising advantages of the transdermal delivery of PMQ to overcome the limitations of oral administration. This study has proven the concept of the improvement of skin permeability of PMQ following the combination of Dermaroller® and transdermal patch. The administration of our approach was considered to be safe and effective not only due to the high permeability of PMQ, but also non-irritant and non-toxicity of this approach to the skin. To further explore the effectiveness of this favorable approach, extensive *in vivo* pharmacokinetic and pharmacodynamic should now be conducted in an appropriate animal model.

4. Conclusion

Transdermal patch of PMQ has been successfully formulated as a single-use patch using HPMC as the main polymeric matrix. The resulting transdermal patch has been evaluated based on physical appearance, uniformity of weight, thickness patch, folding endurance, surface pH, moisture absorption, drug content of determination, moisture content, water absorption ability and water vapor transmission. The evaluation was carried out to ensure the quality of the resulting transdermal patch, showing that all the formulations showed desired characteristics without any interactions between PMQ and all excipients used. *In vitro* and *ex vivo* drug release studies have shown that appropriate drug release was achieved within 24 hours. The use of PEG 400 improved the permeability of PMQ. Importantly, the treated skin with Dermaroller® could significantly increase the permeability of PMQ through the rats' skin *ex vivo*. Furthermore, hemolytic assay exhibited that PMQ in patch did not show potential toxicity. Essentially, *in vivo* irritation study revealed the safety of this combination approach, shown by histopathological evaluation. Following up these findings, appropriate animal studies should be considered.

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Ms. Ref. No.: IJPHARM-D-21-02344

Title: Combination of transdermal patch and solid microneedles for improved transdermal delivery of primaquine

International Journal of Pharmaceutics

Reviewers' comments:

Reviewer #1: The manuscript describes a transdermal drug delivery system designed for malaria treatment. I think that the system described here is simple and can provide advantages for the treatment of malaria. I think that the article is well written and discussed. I have a few minor points that I think that the authors should address before the article can be accepted for publication:

Response to Reviewers

We are very thankful to the reviewers for taking the time to provide helpful comments for improvements to our manuscript. We have addressed each of the reviewers' comments in detail below.

1. The caption for Figure 4 does not provide information about what formulation were 1 and

Response to Reviewer

We thank the reviewer for pointing this out. We have included the complete information in the revised manuscript (Line 343).

2. The discussion of FTIR and DSC can be misleading. When the presence of PMQ peaks in the FTIR of the patch does not mean that there are no interactions between the polymers and the drug. That confirms that the drug is present in the patch. Peak shifts is more indicative of non-covalent interactions. Moreover, if the drug is in amorphous state inside the patch, that suggest some sort of interactions between the polymers and the drug. I think that this should be presented in a more clear way to the reader.

Response to Reviewer

We thank the reviewer for the comments and suggestions. Accordingly, we have re-written our results and discussion, explaining that the presence of PMQ peaks in the FTIR of the patch

suggested the presence of PMQ in the patch formulation. However, it was interesting to note that the peak at 3318 cm^{-1} became broader, which could be due to the formation of hydrogen bonding (Permana et al., 2020) between PMQ and the polymeric matrix, reducing the crystallinity of PMQ. We have explained this in the revised manuscript (Line 337-340).

Additionally, as per the result from DSC analysis, there was an endothermic peak at 202.5°C, indicating the melting point and the crystallinity of pure PMQ. However, this peak was not found in the thermogram of transdermal patch, indicating that the PMQ changed to the amorphous state and was uniformly dispersed in the matrix of the polymeric patch. Additionally, we did not observe additional peaks in the transdermal patch thermogram. The absence of PMQ peak in the transdermal patch is consistent with the results from FTIR evaluation, showing that the possible presence of the hydrogen bonding could reduce the crystallinity of PMQ and form an amorphous state. A previous study has demonstrated similar phenomenon in the formulation of dipyridamole in vascular graft formulations (Domínguez-Robles et al., 2021). We have explained this in the revised manuscript (Line 351-355).

3. I think that the mathematical modelling is not well implemented as all the correlation coefficients are quite low. The authors have biphasic release patterns: a zero order release in the first section of the profiles as the curves are mostly linear. And subsequently, a change in the slope (faster drug delivery). The authors achieved a patch capable of providing a nice zero order release over the first hours and that is really good as no burst release was observed initially. I suggest to apply a zero order model to the linear region only and then describe what could happen to the system to generate that change in the release rate (maybe water absorption?). I think that there is no point on using all these other models as they are not going to present a good fit to this particular release curves.

Response to Reviewer

We thank the reviewer for the comments and suggestions. Indeed, in this study, we observed biphasic release manner. It is important to note that a burst release was not observed in the *in vitro* and *ex vivo* evaluations. This might be due to the slow moisture absorption ability of HPMC, as shown in the determination of moisture absorption ability results, leading to a sustained and slow diffusion rate of PMQ from the patch formulation. After 8 hours, the

permeation profile of F3 combined with 1 mm Dermaroller® showed a zero-order kinetic model with acceptable linearity (R2= 0.9786). Therefore, it could be a favour in transdermal delivery to provide a stable plasma concentration during the application of this patch preparation (Ma et al., 2021). It was also observed that the permeation profile exhibited a biphasic manner after a zero-order kinetic from 8 h to 24 h. During the first 8 h, PMQ in the surface might permeate first due to the water and moisture absorption. Afterwards, the PMQ permeated in a slower rate, providing a sustained release profile. In our study, the formulation of PMQ in transdermal patches could produce a sustained release manner of PMQ over 24 h. It has been previously reported that the uniform distribution of the drug in the patch formulations could also assure the uniform reproducible sustained release of the drug molecules from the patch (Arora and Mukherjee, 2002). Accordingly, the sustained release effect obtained in this study could also be due to the excellent uniformity content of PMQ in the patches. We have clarified this in the revised manuscript (Line 390-395 and Line 399-408)

Reviewer #3: This manuscript designed a formulation that combined a transdermal patch containing primaquine (PMQ) with solid microneedles (Dermaroller ®). Microneedles were expected to help PMQ of the patch to diffuse through skin barriers by creating microchannels. The basic characteristics, permeation study, and irritation and histopathological evaluations in vivo of this system were evaluated. Overall, the whole research work presented a promising combination therapy. This valuable research work can be published after the authors provide more necessary data and revise the manuscript. Some suggestions are as follows.

Response to Reviewers

We are very thankful to the reviewers for taking the time to provide helpful comments for improvements to our manuscript. We have addressed each of the reviewers' comments in detail below.

1. It is recommended that Figure 1 should be added to the supplementary data.

Response to Reviewers

We have removed Figure 1 to the supplementary data.

2. In Figure 4 legend, please indicate what substances 1 and 2 represented.

Response to Reviewer

We thank the reviewer for pointing this out. We have included the complete information in the revised manuscript (Line 343).

3. Line 393, "Figure 5D" should be changed to "Figure 5D, E".

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

4. Given the focus of the combination of solid microneedles and transdermal patches, the advantages of this combination prescription than traditional solid microneedles (free drugs were applied directly after microneedles create microchannels) could not be shown. Relevant experiments need to be supplied.

Response to Reviewer

We thank the reviewer for the comments and suggestions. Therefore, we have performed additional experiment by applying free drugs after microneedles application (Section 2.7, Line 201). The results of this part are discussed in the revised manuscript (Line 409-412).

"The permeation of PMQ from the solution after the administration of Dermaroller® was further evaluated as a comparison to prove the effectiveness of our approach. As shown in Figure 5A, approximately 100% of PMQ permeated the skin only after 3 hours. Accordingly, it was proven that our approach could sustain the release of PMQ."

Additionally, we have also performed *ex vivo* retention skin evaluations (Section 2.7, Line 202-209). The results of this part are discussed in the revised manuscript (Line 412-421).

"Importantly, the concentrations of PMQ deposited in the skin following the administration of PMQ patch and solution, in combination with Dermaroller® with a length of 1 mm were determined. Figure 5B depicts the result of this experiment. As depicted, in solution, PMQ was only deposited after 2 h of administration, with only 165.21 ± 98.11 mg/g skin tissue localized in the skin. On the other hand, following the administration of transdermal patches, the concentration of PMQ deposited in the skin increased over 24 h, resulting in 687.65 ± 118.32 mg/g skin tissue localized in the skin. Accordingly, our results suggested that the administration of PMQ in transdermal patches, combined with Dermaroller®, could improve the permeation profile of PMQ, sustain the release of PMQ, and importantly, enhance the localization of PMQ in the skin over 24 h."

5. There are lots of errors caused by carelessness in the manuscript. And English writing should be further improved. The authors should read over the manuscript carefully and correct all these mistakes.

Response to Reviewer

We thank the Editor for these suggestions. Following the suggestions, we have corrected the sentences. Importantly, we have re-checked the manuscript thoroughly and made significant changes in the English. We believe that the English of the revised manuscript has now been improved.

Some examples are as follows:

Line 59, "This study focuses on the treatment of malaria using PMQ …" should be in the past tense, and the sentence structure was chaotic**.**

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 74-76, one clause and two main clauses appeared in one sentence.

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 76-78, the sentence is hard to understand.

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 99, "from" should be "form".

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 108-109 and line 115, The full name of HPMC was wrong.

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 160, "cm" should be "cm2".

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 188, the unit of temperature is defective.

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 265-266, the presentation of the literature citation was wrong.

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 393, "Figure 5D" should be changed to "Figure 5D, E".

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

Line 400, "Figure x", unclear semantics.

Response to Reviewer

We thank the reviewer for pointing this out. We have corrected this.

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Permana, A.D., Utami, R.N., Courtenay, A.J., Manggau, M.A., Donnelly, R.F., Rahman, L., 2020. 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, 111846.

BUKTI ACCEPTED

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Your Submission

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Ms. Ref. No.: IJPHARM-D-21-02344R1
Title: Combination of transdermal patch and solid microneedles for improved transdermal delivery of primaquine
International Journal of Pharmaceutics

Dear Dr. Permana,

I am pleased to confirm that your paper Combination of transdermal patch and solid microneedles for improved transdermal delivery of primaquine has been accepted for publication in International Journal of Pharmaceutics

Upon transfer of your paper to our production department, your article proof will be created and sent to you for checking. 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.

Thank you for submitting your work to this journal.

With kind regards,

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International Journal of Pharmaceutics

Comments to Authors:

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