BUKTI SUBMIT MANUSKRIP

Universitas Hasanuddin

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

Manuscript # AAPSPT-D-22-00521 Has Been Assigned to Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An ex vivo proof of concept study

1 message

AAPSPT Editorial Office <em@editorialmanager.com> Fri, Jul 8, 2022 at 6:18 AM
Reply-To: AAPSPT Editorial Office <aapsptsubmit@aaps.org>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Dear Dr. Permana,

Your submission entitled "Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An ex vivo proof of concept study" has been assigned the following manuscript number: AAPSPT-D-22-00521.

Your manuscript will now be evaluated by the editor-in-chief. Should your paper be accepted into the peer review process, qualified peer reviewers will be assigned.

The contents of your manuscript are confidential and are not to be copyrighted, submitted, or published elsewhere (including the Internet), in any language, while acceptance by the Journal is under consideration. Please inform all co-authors of this condition.

You will be able to check on the status of your paper by logging on to Editorial Manager as an Author.
The URL is https://www.editorialmanager.com/aapspt/.

Thank you for submitting your work to AAPS PharmSciTech.

Kind regards,

Editorial Office
AAPS PharmSciTech

BUKTI REVIEW DARI REVIEWERS

Universitas Hasanuddin

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

Your Submission AAPSPT-D-22-00521

1 message

AAPSPT Editorial Office <em@editorialmanager.com> Mon, Aug 8, 2022 at 8:50 PM
Reply-To: AAPSPT Editorial Office <aapsptsubmit@aaps.org>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

CC: cbwu2000@yahoo.com, "Nirmayanti Nirmayanti" nirmayanti18n@student.unhas.ac.id, "Alhidayah Alhidayah" alhidayah18n@student.unhas.ac.id, "Jessica Theodor Usman" usmanjt18n@student.unhas.ac.id, "Julika Fajrika Nur" nurjf18n@student.unhas.ac.id, "Muh. Nur Amir" muhnuramir@unhas.ac.id

Dear Dr. Permana

Your manuscript, AAPSPT-D-22-00521, "Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An ex vivo proof of concept study," has been peer reviewed and evaluated editorially; MAJOR revisions have been recommended.

The comments of the Reviewers are appended below, if available. If any attachments are available for this file, an "Attachments" Link will display immediately preceding the reviewer comments.

If you can address all issues raised by the Reviewers, I invite you to resubmit a thoroughly revised manuscript in the next 45 days. If after 45 days you have not resubmitted your revised paper or contacted the editor for an extension of the deadline, your paper will be removed from the Editorial Manager system and you will be required to submit a new manuscript.

Please provide a point-by-point reply that indicates how you have revised your manuscript to address each of the Reviewers' comments, and to my detailed commentary below (if applicable).

Please include these responses as a separate page at the end of the manuscript body document, immediately following the References List. Please, do NOT include your response to comments in the cover letter and do NOT include your response to comments as a supplementary file, as these file types are NOT included in the PDF composition and your comments will NOT be visible to reviewers.

GRAPHICAL ABSTRACTS (optional): If you haven't done so, we urge you to upload a graphical abstract. To do so, when attaching your files for the revision, please select "Graphical Abstract" under the "Select Item Type" dropdown menu. Please then attach your graphical abstract as you would any other file.

NOW MANDATORY:

1. **FUNDING STATEMENT**: If you haven't already, please declare sources of sturdy funding. Please refer to the **author instructions** for more information.

2. **CONFLICT OF INTEREST STATEMENT:** If you haven't already, authors must declare their particular financial relationships to the work described. Please refer to the **author instructions** for more information.

3. **AUTHOR CONTRIBUTIONS**: If you haven't already, please provide a statement outlining each author's contributions to the paper. Please refer to the **author instructions** for more information.

Should you decide not to revise your manuscript in response to a specific comment, please justify your decision in detail. A thorough and reasoned response to the referees' comments will expedite the review process.

Please mark all changes and/or corrections introduced to the manuscript in boldface type within the manuscript body.

IMPORTANT NOTE

AAPS makes an effort to assign new submissions to the editor as quickly as possible for peer review consideration. In order to facilitate this process, we do not always enforce all structural and style requirements specified in our author instructions during the initial submission process.

PLEASE BE ADVISED:

All subsequent revision submissions for this file must adhere to our author instructions. Your revision submission will be reviewed for adherance to but not limited to requirements such as reference style (Vancouver), heading numbering (none allowed) and figure file type. In particular, you will be required to submit your figures as individual files one at a

time; the figures CANNOT be combined into one file. Figure files in PDF, PPT, or Microsoft WORD format will NOT be accepted. Figure files must be submitted as .jpeg, .gif, .tif, .bmp, or .eps file types.

To submit a revised manuscript, go to https://www.editorialmanager.com/aapspt/ and log in as an Author. You will see a menu item called 'Submissions Needing Revision'. You will find your submission record there.

If you have any questions or experience any difficulty, please feel free to email the AAPS Editorial Office at AAPSPTsubmit@aaps.org.

We have appreciated the opportunity to review your manuscript. Thank you for your submission to and support of AAPS PharmSciTech.

Sincerely,

Robert O. Williams III, Ph.D.
Editor-in-Chief
AAPS PharmSciTech

Attachments:

Reviewers' Comments:

Reviewer #1: Abstract
Avoid abbreviations in the abstract (line 3 before line 7)
The methods not clear and not matched with the title and the aim of the manuscript
The results were not matched with conclusion
Introduction
Recheck the idea from line 35-37 add a suitable reference that insure that idea
The aim is not clear
Explain why you should use thermosensitive gel with the microneedles
The idea of using combined poloxamer has no sense
Physical properties of valsatan is preferred to add in the introduction
Methods
The preparation method is very primitive
On what bases, use selected the used ratio
Why you measure The pH of the preparation and you does not use buffer
IN PERMEATION TEST more details about the used skin and MNS should be added
The free drug should be tested in in vitro and in ex vivo release studies
Results and Discussion
Line 157, (met the required) what do you mean?
The discussion is good but the authors should compare the formulation results with free drug and free formula to prove their idea
General noes
The poor English and the many grammar and spelling mistakes require careful attention.
Please highlight the novelty and significance of obtained results.
Compare the results obtained in the current study with those previously studied

Reviewer #2: This manuscript is written very well and very well explained. Field of research is very interesting and have tremendous potential for delivering the drugs that suffer lower bio-availability through oral route. I have some reservations about title of manuscript specially the word combinatorial is not explaining the meaning of study. why valsartan oral bioavailability is less and how this system is improving it, only drug release and permeation studies are not sufficient to prove this claim. Use of these thermosensitive polymers is also not clear, what is benefit of using these polymers here? In vivo studies are very important to confirm bio-availabilty improvement.

Reviewer #4: The article entitled: "Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An ex vivo proof of concept study" needs to be written again with attention to grammar and typo errors.
Page 2, lines 23 -24: In 2010, as much as 31.1% of the global 24 adult population (1.38 billion) had hypertension. Is there a newer reference?
Page 2, lines 27- 28: Oral bioavailability (BA), usually bioavailability is abbreviated as F.
Page 3, line 56: Authors, please correct okyo to Tokyo in: Valsartan (Purity: >98.0%) was obtained from okyo Chemical Industry (Tokyo, Japan).
Page 3, line 76: "thermosensitive gel, equal to 10 mg of VALS (0,1 mL), was pipetted out". Does this phrase mean, a concentration of 10 mg VALS/0.1 mL was pipetted?
Page 3, lines 76-78: Was there any standard curve run? What is the manufacturing company for the UV/Visible used?

In general, methodology needs more details and description.
In vivo animal experiments: There is no mention about ethics and the guideline followed in using experimental animals. How was the skin obtained from the rat and prepared for an in vivo experiment? The experiment needs more details about the skin prepared from the rat
Effect of poloxamer concentration in in vitro release and permeation of VALS: What method(s) was used to quantify and determine the amount of the drug permeated and/or released from each formula in vitro and in vivo? More details are needed.
Please give a frank account of the strengths and weaknesses of the article: The article is weak and not suitable for publication.

BUKTI SUBMIT HASIL REVIEW

Universitas Hasanuddin

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

Submission Confirmation for AAPSPT-D-22-00521R1

1 message

AAPSPT Editorial Office <em@editorialmanager.com> Thu, Sep 1, 2022 at 1:11 AM
Reply-To: AAPSPT Editorial Office <aapsptsubmit@aaps.org>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Re: Manuscript AAPSPT-D-22-00521R1
Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An in vivo proof of concept study
AAPS PharmSciTech

Dear Dr. Permana,

AAPS PharmSciTech has received your revised submission.

You may check the status of your manuscript by logging onto Editorial Manager at https://www.editorialmanager.com/aapspt/.

Kind regards,

Editorial Office
AAPS PharmSciTech

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

Pharmaceutical Research

July 5, 2022

Dear Sir/Madam,

I would like to submit our original manuscript entitled "**Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An *ex vivo* proof of concept study"** for publication in ***Pharmaceutical Research.***

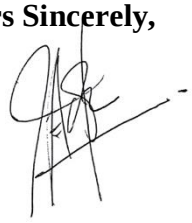
This study summarizes the novel thermosensitive hydrogels transdermal delivery of valsartan in combination with solid microneedles. This study intended to determine the effect of poloxamer concentration on the characteristics and physical properties of valsartan thermosensitive gel preparation and its combination with solid MNs. Valsartan is currently used orally but has a low bioavailability of only 10-35%, due to the first pass effect. To solve this problem, another route that can be used is the transdermal route, which valsartan is a promising drug candidate in this delivery system. However, one of the drawbacks of transdermal preparations is their low penetrability due to the presence of a *stratum corneum* layer on the skin. Accordingly, a permeation enhancer is needed, one of which is solid microneedle (MNs). The thermosensitive gel formula was made using Poloxamer 407 and Poloxamer 188 in various ratios. valsartan thermosensitive gels were evaluated for their gelation temperature, pH values, drug content, spreadibility, viscosity, rheological properties, *in vitro* drug release, *in vitro* permeation and *ex vivo* permeation. The results presented two formula showed required characteristic for transdermal administration. Based on the permeation test with and without MNs, it was found that the use of MNs increased valsartan permeation and increased MNs needle length also increased valsartan permeation. The concentration of poloxamer was able to affect the properties of the hydrogels and the use of

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

solid microneedles improved the transdermal delivery of valsartan. *In vivo* studies should now be performed.

This manuscript was previously rejected by Journal of Pharmaceutical Innovation. However, we believe that this finding will be of interest to the scientists working on the use of polymeric materials in pharmaceutical technology, transdermal delivery pharmaceutics, polymer technology and correlation *in vitro* and *ex vivo* evaluations. This manuscript has not been previously published in any language anywhere and that it is not under simultaneous consideration by another journal. We appreciate your attention. We hope you will now consider publishing our research in ***Pharmaceutical Research*** 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

AAPSPT-D-22-00521, "Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An ex vivo proof of concept study,"

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. Additionally, as we have performed *in vivo* study, we changed our title to Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An *in vivo* proof of concept study

Reviewers' Comments:

Reviewer #1: Abstract

Avoid abbreviations in the abstract (line 3 before line 7)

Response:

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

The methods not clear and not matched with the title and the aim of the manuscript

Response:

We thank the reviewer for the comments. As a result, we have changed the aim of the manuscript in order to match with the title. Additionally, we have also improved the explanation of the methods and performed additional study, including *in vivo* study to support the title.

The results were not matched with conclusion

Response:

We thank the reviewer for the comments. As mentioned previously, we also improved the explanation of the methods and performed additional study, including *in vivo* study to support the title. We have also discussed the results to support the conclusion of the study.

Introduction

Recheck the idea from line 35-37 add a suitable reference that insure that idea

Response:

We thank the reviewer for pointing this out. We have rephrased the sentence to clarify the idea.

The aim is not clear

Response:

We thank the reviewer for the comments. We have changed the aim of the manuscript in order to match with the title.

Explain why you should use thermosensitive gel with the microneedles

Response:

We thank the reviewer for the comments. The major issue in the transdermal delivery system is the presence of *stratum corneum* (SC), the lipophilic layer of skin. It has been reported that VALS has a log partition coefficient of 5.8 (Himawan *et al.*, 2022), showing its lipophilicity which could decrease its ability to reach the dermal circulation for further systemic absorption (Khan *et al.*, 2019). One of the strategies adopted to bypass the SC barrier is using solid microneedles (MNs) as physical penetration enhancers attributed to their painless properties and insignificant infection risk at the application site (Ahmed *et al.*, 2019). Among several types of solid MNs, Dermarollers® has been widely used to improve transdermal delivery of numerous active pharmaceutical ingredients (Badran, Kuntsche and Fahr, 2009; Ahmed *et al.*, 2019; Ananda *et al.*, 2021). MNs as a pretreatment could create micropores in the skin and close by ~48 h and would need to be repeated. Therefore, a suitable delivery system is required in order to reduce the application frequency for patient compliance. One of the transdermal delivery systems is thermosensitive gel, the formulation that changes from liquid to gel under an increase in temperature. This approach could potentially be used to reduce the frequency of MNs application because it would quickly enter into micropores when applied and change into gel slowly, which would create in situ gel depot in the micropores that would continue to deliver

drug even if the micropores close (Tobin, Fiegel and Brogden, 2021). These have been included in the revised manuscript.

The idea of using combined poloxamer has no sense

Response:

We thank the reviewer for the comments. The most frequently used thermosensitive polymer in gel preparations is Poloxamer 407. However, a Poloxamer 407 solution with more than 20% concentration would form a hydrogel at ambient temperature and reduce the gelation temperature of the preparation to <25°C so that it can form a gel at ambient temperature (Enggi *et al.*, 2021; Asma *et al.*, 2022). Several studies have shown the limitation of the single use of Poloxamer 407 (Din *et al.*, 2015; Soliman, Fetih and M, 2016; Tuğcu-Demiröz, 2017; Permana, Nurul, *et al.*, 2021; Zhang *et al.*, 2021), showing the necessity to combine this thermosensitive polymer with another polymer. To overcome this, the addition of another type of Poloxamer, Poloxamer 188 has been previously used to modify the gelation temperature of the preparation. It was reported that combining the two types of Poloxamer could potentially modulate the gelation temperature of the preparations (Chen *et al.*, 2013). These have been included in the revised manuscript.

Physical properties of valsatan is preferred to add in the introduction

Response:

We thank the reviewer for the suggestion. Valsartan (VALS) is a selective angiotensin II type 1 receptor blocker that is used orally. Oral bioavailability (F) of VALS is 10-35% and shows a first-pass effect and low absorption through gastrointestinal. C_{max} and AUC VALS can be diminished by food intake, which may reduce the pharmacological effects (Bhosale and Avachat, 2013). Accordingly, it is critical to develop an alternative delivery route for VALS. It has been previously reported that transdermal administration could be potentially utilized to overcome several issues of the oral route (Alkilani, McCrudden and Donnelly, 2015; Permana *et al.*, 2019; Permana, McCrudden and Donnelly, 2019). Regarding its properties, VALS has a low molecular weight of 435.5 Da, melting point of 116-117°C, pKa of 4.73, mean biological half-life of 7.5 h and log

partition coefficient of 4.5 (Park *et al.*, 2010; Yan *et al.*, 2012; Xu *et al.*, 2016; Sandip *et al.*, 2018; Santinon *et al.*, 2021). Additionally, VALS is a candidate that promising for transdermal drug delivery and there are no reports about skin irritation caused by VALS (Ahad, Aqil and Ali, 2014). These have been included in the revised manuscript.

Methods

The preparation method is very primitive

Response:

We thank the reviewer for the comment. However, the cold method in the preparation of thermosensitive hydrogel have been widely applied in numerous studies (Wei *et al.*, 2020; Alhidayah *et al.*, 2021; Argenta *et al.*, 2021; Enggi *et al.*, 2021; Permana, Nurul, *et al.*, 2021; Permana, Utomo, *et al.*, 2021; Asma *et al.*, 2022). Therefore, we believe that this method is a standard method to prepare the preparation.

On what bases, use selected the used ratio

Response:

We thank the reviewer for the question. Initially, the concentration used was selected according the concentration of Poloxamer which has been generally used to prepare thermosensitive preparation. Following the addition of VALS, it was observed that the properties also changed. Therefore, we modified the concentration as per Table 1 in our manuscript to obtain the formulation with desired characteristics.

Why you measure The pH of the preparation and you does not use buffer

Response:

We thank the reviewer for the question. The pH test was carried out to investigate whether the skin could well tolerate the pH of the transdermal gel preparation. Preparations with a pH outside this range could potentially cause problems, such as irritation, itching or burning of the skin (Alberti *et al.*, 2005). Generally, skin can tolerate the administration of the topical preparation

with the pH values ranging from 4-7 (Kim and Baek, 2014). Therefore, although we did not use buffer, the determination of pH was crucial.

IN PERMEATION TEST more details about the used skin and MNS should be added

Response:

We thank the reviewer for pointing this out. We have added more details about this part in the revised manuscript.

The free drug should be tested in in vitro and in ex vivo release studies

Response:

We thank the reviewer for the suggestion. We have performed additional studies for free drug and have included the results in the revised manuscript.

Results and Discussion

Line 157, (met the required) what do you mean?

Response:

We thank the reviewer for the question. We have rephrased the sentence to clarify the idea. Generally, skin can tolerate the administration of the topical preparation with the pH values ranging from 4-7 (Kim and Baek, 2014). As shown in Table 2, all the measured pH was found to be in the range

The discussion is good but the authors should compare the formulation results with free drug and free formula to prove their idea

Response:

We thank the reviewer for the suggestion. We have performed additional studies for free drug and have included the results in the revised manuscript.

General

The poor English and the many grammar and spelling mistakes require careful attention.

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.

Please highlight the novelty and significance of obtained results.

Response:

We thank the reviewer for the suggestion. We have added this in the revised manuscript. The results showed that compared to oral administration, transdermal delivery could improve the bioavailability of VALS, indicated by the improvement of AUC values. The highest AUC value was achieved after the combination of thermosensitive hydrogel with MNs, showing the value of 17.26 ± 1.98 µg/mL*h. This value was significantly higher ($p < 0.05$) compared to AUC values of oral administration and thermosensitive hydrogel groups. Moreover, the residence time of VALS was also significantly improved through transdermal delivery, showing the controlled release manner of VALS. As discussed previously, the pores created by MNs would allow the effective permeation of the thermosensitive hydrogels. Due to the thermosensitive properties, the transformation of the formulation into gel in the body temperature would be beneficial to sustain the release of VALS to the systemic circulation from the skin. Accordingly, the combination of thermosensitive and MNs offers two main benefits. First, it could improve the bioavailability of VALS. Second, this approach could sustain the *in vivo* release of VALS. This could potentially reduce the administration frequency of the treatment, leading the patient compliance during the hypertension therapy. Overall, the results obtained in this study served as a proof of concept, showing that the permeation of VALS through the skin from thermosensitive hydrogel could potentially be improved using solid MNs. This could be beneficial in the treatment of hypertension.

Compare the results obtained in the current study with those previously studied

Response:

We thank the reviewer for the suggestion We have compared our results with some previous studies in the revised manuscript.

Reviewer #2: This manuscript is written very well and very well explained. Field of research is very interesting and have tremendous potential for delivering the drugs that suffer lower bio-availability through oral route. I have some reservations about title of manuscript specially the word combinatorial is not explaining the meaning of study. why valsartan oral bioavailability is less and how this system is improving it, only drug release and permeation studies are not sufficient to prove this claim. Use of these thermosensitive polymers is also not clear, what is benefit of using these polymers here? In vivo studies are very important to confirm bio-availabilty improvement.

Response:

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 also improved the explanation of the methods and performed additional study, including *in vivo* study to support the title. We have also discussed the results to support the conclusion of the study, showing the improvement of bioavailability compared to oral administration.

Reviewer #4: The article entitled: "Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An ex vivo proof of concept study" needs to be written again with attention to grammar and typo errors.

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.

Page 2, lines 23 -24: In 2010, as much as 31.1% of the global 24 adult population (1.38 billion) had hypertension. Is there a newer reference?

Response:

We thank the reviewer for pointing out. We have updated the data of hypertension using a newer reference in the revised manuscript.

Page 2, lines 27- 28: Oral bioavailability (BA), usually bioavailability is abbreviated as F.

Response:

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

Page 3, line 56: Authors, please correct okyo to Tokyo in: Valsartan (Purity: >98.0%) was obtained from okyo Chemical Industry (Tokyo, Japan).

Response:

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

Page 3, line 76: "thermosensitive gel, equal to 10 mg of VALS (0,1 mL), was pipetted out". Does this phrase mean, a concentration of 10 mg VALS/0.1 mL was pipetted?

Response:

We thank the reviewer for pointing this out. We apologize for the confusion. We have revised the sentence to "VALS thermosensitive gel (0.1 mL), equal to 10 mg of VALS, was pipetted out" to avoid the confusion to the reader.

Page 3, lines 76-78: Was there any standard curve run? What is the manufacturing company for the UV/Visible used?

Response:

We thank the reviewer for the question. The absorbance of the sample was measured at 230.2 nm with a UV-Vis spectrophotometer (Dynamica, HALO XB-10). The concentration of VALS was determined by plotting the absorbance values to the calibration curve prepared from the standard solution of VALS in the concentration range of 0.5 µg/mL – 32 µg/mL. We have included these in the revised manuscript.

In general, methodology needs more details and description.

Response:

We thank the reviewer for the suggestion. As a result, we have added more details in the methods in the revised manuscript. We believe that our manuscript is now clear.

In vivo animal experiments: There is no mention about ethics and the guideline followed in using experimental animals. How was the skin obtained from the rat and prepared for an in vivo experiment? The experiment needs more details about the skin prepared from the rat

Response:

We thank the reviewer for the suggestion. The abdominal skin of Female Sprague-Dawley rats was shaven and used as a biological membrane for the study. Initially, the hair of the rats was

shaved and the hair removal cream was applied to the shaved area. The rats were sacrificed and then the skins were collected. The skin was washed with distilled water and equilibrated in PBS before the experiment. The *ex vivo* experiment was approved by the Health Ethical Committee at the Faculty of Medicine, Hasanuddin University, Indonesia.

The *in vivo* investigation was conducted to investigate the penetrability of VALS from thermosensitive hydrogels after the application of solid MNs. The study was performed on healthy male Wistar rats weighing 213.08 ± 14.17 g. All the animals were acclimatized for one week in the conditions of the laboratory. The *in vivo* experiment was approved by the Health Ethical Committee at the Faculty of Medicine, Hasanuddin University, Indonesia. There were three groups in this study: the first group received thermosensitive gels containing VALS after the administration of solid MNs, the second group received thermosensitive gels containing VALS without the administration of solid MNs and the third group received oral administration of VALS. All group received VALS with a dose of 10 mg/kg of body weight. At predetermined interval times, the bloods were collected, and the plasma samples were separated for further analysis.
All these explanations have been added in the revised manuscript.

Effect of poloxamer concentration in in vitro release and permeation of VALS: What method(s) was used to quantify and determine the amount of the drug permeated and/or released from each formula in vitro and in vivo? More details are needed.

Response:

We thank the reviewer for the suggestion. We have added detail information about all of the required details in the revised manuscript.

Please give a frank account of the strengths and weaknesses of the article: The article is weak and not suitable for publication.

Response:

We thank the reviewer for the comment. We have addressed each of the reviewers' comments in detail. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript. We believe now that the revised manuscript is suitable for publication.

REFERENCES:

Ahad, A., Aqil, M. and Ali, A. (2014) 'Investigation of antihypertensive activity of carbopol valsartan transdermal gel containing 1,8-cineole', *International Journal of Biological Macromolecules*, 64, pp. 144–149. doi: 10.1016/j.ijbiomac.2013.11.018.

Ahmed, K. S. *et al.* (2019) 'Derma roller® microneedles-mediated transdermal delivery of doxorubicin and celecoxib co-loaded liposomes for enhancing the anticancer effect', *Materials Science and Engineering C*, 99(February), pp. 1448–1458. doi: 10.1016/j.msec.2019.02.095.

Alberti, I. *et al.* (2005) 'Pharmaceutical development and clinical effectiveness of a novel gel technology for transdermal drug delivery', *Expert Opinion on Drug Delivery*, 2(5), pp. 935–950. doi: 10.1517/17425247.2.5.935.

Alhidayah *et al.* (2021) 'Validation of spectrophotometric method for quantification of cefazoline in simulated tear fluid and porcine ocular tissue from thermosensitive-mucoadhesive in situ ocular gel preparation', *Chemical Data Collections*, 36(November), p. 100793. doi: 10.1016/j.cdc.2021.100793.

Alkilani, A. Z., McCrudden, M. T. C. and Donnelly, R. F. (2015) 'Transdermal drug delivery: Innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum', *Pharmaceutics*, 7(4), pp. 438–470. doi: 10.3390/pharmaceutics7040438.

Ananda, P. W. R. *et al.* (2021) 'Combination of transdermal patches and solid microneedles for

improved transdermal delivery of primaquine', *International Journal of Pharmaceutics*, 609(August), p. 121204. doi: 10.1016/j.ijpharm.2021.121204.

Argenta, D. F. *et al.* (2021) 'Thermosensitive hydrogels for vaginal delivery of secnidazole as an approach to overcome the systemic side-effects of oral preparations', *European Journal of Pharmaceutical Sciences*, 159(October 2020). doi: 10.1016/j.ejps.2021.105722.

Asma, N. *et al.* (2022) 'New HPLC-UV analytical method for quantification of metronidazole: Application to ex vivo ocular kinetic assessments following the administration of thermosensitive ocular in situ gel', *Microchemical Journal*, 172(PA), p. 106929. doi: 10.1016/j.microc.2021.106929.

Badran, M. M., Kuntsche, J. and Fahr, A. (2009) 'Skin penetration enhancement by a microneedle device (Dermaroller®) in vitro: Dependency on needle size and applied formulation', *European Journal of Pharmaceutical Sciences*, 36(4–5), pp. 511–523. doi: 10.1016/j.ejps.2008.12.008.

Bhosale, S. S. and Avachat, A. M. (2013) 'Design and development of ethosomal transdermal drug delivery system of valsartan with preclinical assessment in Wistar albino rats', *Journal of Liposome Research*, 23(2), pp. 119–125. doi: 10.3109/08982104.2012.753457.

Chen, J. *et al.* (2013) 'Mechanical, rheological and release behaviors of a poloxamer 407/poloxamer 188/carbopol 940 thermosensitive composite hydrogel', *Molecules*, 18(10), pp. 12415–12425. doi: 10.3390/molecules181012415.

Din, F. U. d. *et al.* (2015) 'Novel dual-reverse thermosensitive solid lipid nanoparticle-loaded hydrogel for rectal administration of flurbiprofen with improved bioavailability and reduced initial burst effect', *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik e.V*, 94, pp. 64–72. doi: 10.1016/j.ejpb.2015.04.019.

Enggi, C. K. *et al.* (2021) 'Development of thermosensitive and mucoadhesive gels of cabotegravir for enhanced permeation and retention profiles in vaginal tissue: A proof of concept study', *International Journal of Pharmaceutics*, 609(September), p. 121182. doi: 10.1016/j.ijpharm.2021.121182.

Himawan, A. *et al.* (2022) 'A novel in vitro approach to investigate the effect of food intake on

release profile of valsartan in solid dispersion-floating gel in-situ delivery system', *European Journal of Pharmaceutical Sciences*, 168(November 2021), p. 106057. doi: 10.1016/j.ejps.2021.106057.

Khan, S. *et al.* (2019) 'Evaluation of microneedles-assisted in situ depot forming poloxamer gels for sustained transdermal drug delivery', *Drug Delivery and Translational Research*, 9(4), pp. 764–782. doi: 10.1007/s13346-019-00617-2.

Kim, M. S. and Baek, I. H. (2014) 'Fabrication and evaluation of valsartan-polymer- surfactant composite nanoparticles by using the supercritical antisolvent process', *International journal of nanomedicine*, 9, pp. 5167–5176. doi: 10.2147/IJN.S71891.

Park, Y. J. *et al.* (2010) 'Improved pH-independent dissolution and oral absorption of valsartan via the preparation of solid dispersion', *Archives of Pharmacal Research*, 33(8), pp. 1235–1240. doi: 10.1007/s12272-010-0814-0.

Permana, A. D. *et al.* (2019) 'Solid lipid nanoparticle-based dissolving microneedles: A promising intradermal lymph targeting drug delivery system with potential for enhanced treatment of lymphatic filariasis', *Journal of Controlled Release*, 316, pp. 34–52.

Permana, A. D., Utomo, E., *et al.* (2021) '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 Applied Materials and Interfaces*, 13(15), pp. 18128–18141. doi: 10.1021/acsami.1c03422.

Permana, A. D., Nurul, R., *et al.* (2021) 'Thermosensitive and mucoadhesive in situ ocular gel for effective local delivery and antifungal activity of itraconazole nanocrystal in the treatment of fungal keratitis', *International Journal of Pharmaceutics*, 602, p. 120623.

Permana, A. D., McCrudden, M. T. C. and Donnelly, R. F. (2019) 'Enhanced intradermal delivery of nanosuspensions of antifilariasis drugs using dissolving microneedles: A proof of concept study', *Pharmaceutics*, 11(7), p. 346.

Sandip, S. *et al.* (2018) 'Solid dispersion of valsartan for solubility improvement using β-cyclodextrin', *MOJ Bioequivalence & Bioavailability*, 5(6). doi: 10.15406/mojbb.2018.05.00121.

Santinon, C. *et al.* (2021) 'Modification of valsartan drug release by incorporation into

sericin/alginate blend using experimental design methodology', *European Polymer Journal*, 153(April), p. 110506. doi: 10.1016/j.eurpolymj.2021.110506.

Soliman, G. M., Fetih, G. and M, A. A. (2016) *Thermosensitive Bioadhesive Gels for The Vaginal Delivery of Sildenafil Citrate : In Vitro Characterization and Clinical Evaluation in Women Using Clomphene Citrate for Induction of Ovulation*, *Drug Development and Industrial Pharmacy*. Taylor & Francis. doi: 10.1080/03639045.2016.1254239.

Tobin, K. V., Fiegel, J. and Brogden, N. K. (2021) 'Thermosensitive gels used to improve microneedle-assisted transdermal delivery of naltrexone', *Polymers*, 13(6), pp. 1–15. doi: 10.3390/polym13060933.

Tuğcu-Demiröz, F. (2017) 'Development of in situ poloxamer-chitosan hydrogels for vaginal drug delivery of benzydamine hydrochloride: Textural, mucoadhesive and in vitro release properties', *Marmara Pharmaceutical Journal*, 21(4), pp. 762–770. doi: 10.12991/mpj.2017.3.

Wei, Y. *et al.* (2020) 'Comparison of thermosensitive in situ gels and drug-resin complex for ocular drug delivery: In vitro drug release and in vivo tissue distribution', *International Journal of Pharmaceutics*, 578(February), p. 119184. doi: 10.1016/j.ijpharm.2020.119184.

Xu, W. J. *et al.* (2016) 'Enhanced dissolution and oral bioavailability of valsartan solid dispersions prepared by a freeze-drying technique using hydrophilic polymers', *Drug Delivery*, 23(1), pp. 41–48. doi: 10.3109/10717544.2014.903012.

Yan, Y. D. *et al.* (2012) 'Novel valsartan-loaded solid dispersion with enhanced bioavailability and no crystalline changes', *International Journal of Pharmaceutics*, 422(1–2), pp. 202–210. doi: 10.1016/j.ijpharm.2011.10.053.

Zhang, T. *et al.* (2021) 'Novel glucosamine-loaded thermosensitive hydrogels based on poloxamers for osteoarthritis therapy by intra-articular injection', *Materials Science and Engineering C*, 118(July 2020), p. 111352. doi: 10.1016/j.msec.2020.111352.

Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An *ex vivo* proof of concept study

Nirmayanti, Alhidayah, Jessica Theodor Usman, Julika Fajrika Nur, Muh. Nur Amir, Andi Dian Permana*,
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

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

ETHICAL DECLARATION

We have no ethical issue to declare

ABSTRACT

Purpose: This study intended to determine the effect of poloxamer concentration on the characteristics and physical properties of valsartan thermosensitive gel preparation and its combination with solid MNs. Valsartan is currently used orally but has a low bioavailability of only 10-35%, due to the first pass effect. To solve this problem, another route that can be used is the transdermal route, which valsartan is a promising drug candidate in this delivery system. However, one of the drawbacks of transdermal preparations is their low penetrability due to the presence of a *stratum corneum* layer on the skin. Accordingly, a permeation enhancer is needed, one of which is solid microneedle (MNs).

Methods: The thermosensitive gel formula was made using Poloxamer 407 and Poloxamer 188 in various ratios. valsartan thermosensitive gels were evaluated for their gelation temperature, pH values, drug content, spreadibility, viscosity, rheological properties, *in vitro* drug release, *in vitro* permeation and *ex vivo* permeation.

Results: The results presented two formula showed required characteristic for transdermal administration. Based on the permeation test with and without MNs, it was found that the use of MNs increased valsartan permeation and increased MNs needle length also increased valsartan permeation. The combination with the highest permeation was produced by 1.55 mm MNs with the amount of drug permeated is 2.27 ± 0.01 mg.

Conclusion: The concentration of poloxamer was able to affect the properties of the hydrogels and the use of solid microneedles improved the transdermal delivery of valsartan. *In vivo* studies should now be performed.

Keywords: valsartan, transdermal thermosensitive gel, solid microneedle

1. Introduction

Hypertension is a disease that is defined as persistently elevated arterial blood pressure (BP) ≥140 mmHg and/or diastolic BP ≥90 mmHg. Hypertension is one of the primary risk factors for cardiovascular disease [1]. Management of hypertension has become international health priority [2]. In 2010, as much as 31.1% of the global adult population (1.38 billion) had hypertension. Globally, the prevalence of hypertension is increases in exposure to lifestyle risk factors [3]. Hypertension, therewith pre-hypertension and other hazardously high blood pressure is liable for 8.5 million deaths from stroke and other vascular disease worldwide [4].

Valsartan (VALS) (Fig. 1) is a selective angiotensin II type 1 receptor blocker that is used orally. Oral bioavailability (BA) of VALS is 10-35% and shows first-pass effect and low absorption through GI. C_{max} and AUC VALS can diminished by food intake which may reduce the pharmacological effect [5]. VALS has low molecular weight of 435.5 Da and melting point of 116-117^{o}C, pK_a of 4.73, mean biological half-life of 7.5 h and log partition coefficient 4.5. VALS is a candidate that promising for transdermal drug delivery and there are no reports about skin irritation caused by VALS [6]

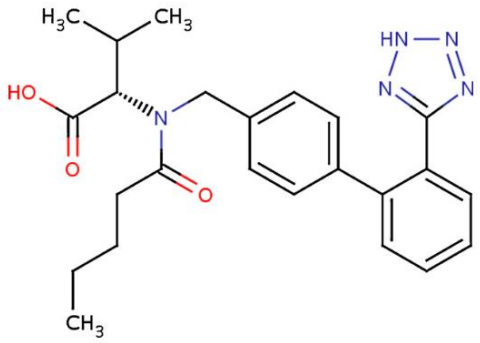


Figure 1. Chemical structure of valsartan

The major issue of transdermal delivery system is the presence *stratum corneum* (SC), the lipophilic layer of skin. VALS has large log partition coefficient (4.5) that makes it lipophilic and would make the permeation across the SC decrease [7]. One of strategies adopted to bypass the SC barrier is using solid microneedles (MNs) as physical penetration enhancer attributed to their painless properties and insignificant infection risk at the application site [8]. MNs as a pretreatment could create micropores in the skin and close by ~48 h and would need to be repeated. One of transdermal delivery system is thermosensitive gel, the formulation that changes from liquid to gel under the increase of temperature. This approach could potentially be used to reduce the frequency of MNs application because would quickly enter into micropores when applied and change into gel slowly which would create in situ gel depot in the micropores that would continue to deliver drug even the micropores close [9]

The most frequently used thermosensitive polymer in gel preparations is Poloxamer 407. However, a Poloxamer 407 solution whose concentration is more than 20% would form a hydrogel at ambient temperature and would reduce the gelation temperature of the preparation to <25 ^{o}C so that it can form a gel at ambient temperature. room temperature. To overcome this, Poloxamer 188 is used to modify the gelation temperature of the preparation, because the combination of the two types of poloxamer will increase the gelation temperature of the preparation [10].

To the best of our knowledge, there have been no studies developing valsartan in the thermosensitive hydrogels for transdermal delivery and its combination with microneedle delivery system. In this study, we developed thermosensitive hydrogel containing valsartan and evaluated their physical and thermosensitive properties. The selected formulation was then combined with solid microneedles with different length and were evaluated for their permeation profile using a rat skin model.

2. Materials and Methods

2.1. Materials

Valsartan (Purity: >98.0%) was obtained from okyo Chemical Industry (Tokyo, Japan). Poloxamer 407 and 188 were kindly provided by BASF Indonesia, Jakarta). Solid microneedles (Dermarollers®) were purchased from SQY® (Guangdong, China). Other compounds used in this study were analytical grade

2.2. Design of Formulation

The poloxamer solution was prepared using the cold method by slowly adding the poloxamer combination that weighed accurately as shown in Table 1 into cold distilled water (4ºC) and then stirring constantly to form a poloxamer solution. The poloxamer solution was then stored in the refrigerator overnight until a clear solution is formed. Amount of VALS was dissolved in ethanol 95% and dissolved in already-prepared poloxamer solution at 4ºC [6,7]

Table 1. Design of VALS thermosensitive-gel formulation

Ingredients % (w/w)	F1	F2	F3	F4	F5
Valsartan	1	1	1	1	1
Ethanol 95%	10	10	10	10	10
Poloxamer 407	17	17	17	18	16
Poloxamer 188	1	3	5	2	4
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

2.3. Evaluation of physical properties

2.3.1. Gelation temperature

Gelation temperature was determined by placing 2 mL of each formula into test tube [11,12]. The test tube was put into water bath at 20ºC and then the test tube were rotated at 90º every 1ºC increase in temperature until the temperature reached 65ºC. The gelation temperature recorded was the temperature at which the gel did not move when the test tube was rotated. The test was run in triplicate.

2.3.2. pH determination

The pH of VALS thermosensitive gel was measured using a digital pH meter, and the test was performed in triplicate [13].

2.3.3. Drug content

VALS thermosensitive gel, equal to 10 mg of VALS (0,1 mL), was pipetted out. It was diluted with ethanol and the absorbance of the mixture was measured at 230.2 nm with UV-Vis spectrophotometer. The drug content was calculated against the absorbance of control VALS solution at 230.2 nm. [14].

2.3.4. Spreadibility

The spreadability of VALS thermosensitive gel was determined at gelling state by placed amount of 0.5 g gel in a 2 cm diameter circle premarked on a glass piece over a second glass plate was placed. A weight of 500 g was rested on the top of glass piece for 5 min. The diameter increased appropiate to the spreading of the preparation was record in triplicate [6]**.**

2.3.5. Viscosity and rheological properties

Viscosity of VALS thermosensitive gel determined by using Viskometer (Brookfield, USA) in various temperature (4 ± 0.1 °C, 25 ± 0.1 °C and 35 ± 0.1 °C) by put each formulation on the lower plate of the apparatus and the viscometer was run using spindle number 63 at 60 rpm. The rheological behavior was determined by calculated viscosity against the various velocity (rpm) of VALS thermosensitive gel [14] [15].

2.4. *In vitro* release study

The *in vitro* release of VALS thermosensitive gels was measured by dissolution model without membrane. Amount of 5 g VALS thermosensitive gel was taken and put in a glass vial and equilibrating to the gelation temperature at 37 ± 1 °C. Total of 2.5 ml of PBS was flowed smoothly on the upper gels without interfere the gel surface. The number of 1 mL were collected at regular time intervals for release quantification. For maintaining the sink condition, fresh PBS medium (1 ml) was added to the glass vials each time. The absorbance of samples were measured using a UV-Vis spectrophotometer at 231.4 nm and the drug contents was calculated [7].

2.5. *In vitro* permeation study

Franz diffusion cell was used to assess the *in vitro* permeation of VALS. Cellophane membrane used as membrane for the study. The receptor compartment was fill up with PBS (pH 7.4) and also filled with magnetic bead for stirring purpose. The membrane was placed in between the receptor and the donor compartment. The cell was hold at 37 ± 1◦C and agitated by magnetic stirrer at 90 rpm. Amount of 1 g gel preparation was placed to the donor compartment. About 1 mL sample was took at the regular time intervals. After each time, equal volume of fresh PBS (pH 7.4) that be heated to 37 ± 1◦C was added in the receptor compartment to keep the sink conditions. The samples absorbance was measured using UV-Vis spectrophotometer at 231.4 nm [8,14].

2.6. *Ex vivo* permeation study

Similarly, Franz diffusion cell was also used to investigate the *ex vivo* permeation study [17–19]. Abdominal skin of Female Sprague-Dawley rats was shaven and used as biological membrane for the study. The receptor compartment was filled up with PBS (pH 7.4) and magnetic bead for stirring purpose. In an attempt to evaluate the effect of solid MNs treatment on the penetration through the skin, the skin sample was pretreated with a Dermaroller® before it was placed onto the donor compartment. The skin was placed in between the receptor and the donor compartment. The cell was maintained at 37 ± 1◦C and agitated by magnetic stirrer at 200 rpm. Amount of 1 g of gel was placed to the donor compartment. About 1 mL sample was took at the regular time intervals and replace by 1 mL fresh PBS (pH 7.4) heated to 37 ± 1◦C to keep the sink conditions. The samples absorbance was assessed using UV-Vis spectrophotometer at 231.4 nm.

2.7 Statistical analysis

All the data were presented as means ± standard deviation (SD) of the mean. SD of the results was measured using Microsoft® Excel® 2010 (Microsoft Corporation, Redmond, USA). The statistical analysis was performed

using GraphPad Prism® version 5.0.3 (GraphPad Software, San Diego, California, USA). The $p < 0.05$ indicated a significant difference in all cases.

3. Result and Discussion

3.1. VALS thermosensitive gel formulation

In this study, a thermosensitive transdermal gel formulation of VALS was carried out and several physical characteristics, release profile and drug permeation in rat skin on *ex vivo* study were performed. VALS thermosensitive gel was made in five different formulas containing a combination of Poloxamer 407 and Poloxamer 188 as thermosensitive polymers. The representative image of the resulting preparation can be seen in **Fig. 2**. The results of organoleptic observations showed that all the thermosensitive gel preparations were slightly clear with a bit smell of alcohol. This is accordance with previous research that gels should appear transparent [20]. In addition, the resulting preparation of thermosensitive gel was in the liquid form at room temperature and gel form when the gelation temperature was reached.

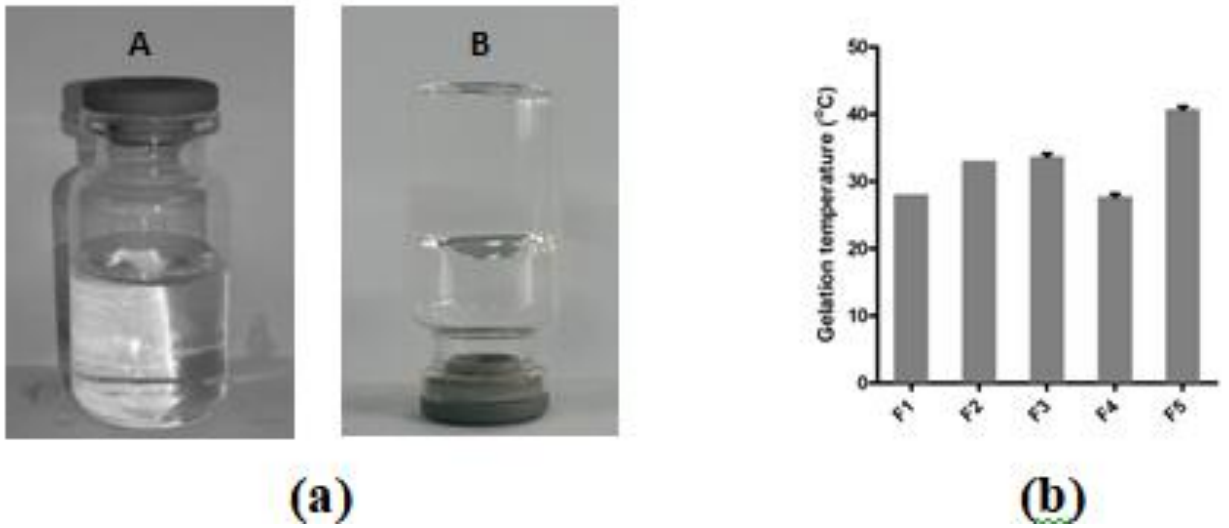


Fig. 2. (a) VALS thermosensitive gel (A) in room temperature (B) in body temperature; (b) gelation temperature of VALS thermosensitive gel (mean ± SD, n=3).

3.2. Evaluation of physical properties

3.2.1. Effect of poloxamer concentration on gelation temperature

Gelation temperature testing was carried out to obtain temperature required by VALS thermosensitive transdermal gel to transform into gel form. The thermosensitive transdermal gel should have a gelation temperature similar to the physiological skin temperature, which is 32 ± 1 °C. So, when transdermal gel is applied, the preparation would become gel at the skin [21]. The gelation temperature of VALS thermosensitive transdermal gel can be seen in **Fig. 2.** The test results show that the gelation temperature of each formula obtained were in the range of 27.67°C - 40.67°C. The results showed that F2 and F3 had gelation temperatures that were most similar to the physiological skin temperature Thermosensitive gels with the same concentration of Poloxamer 407 and Poloxamer 188 showed that

gelation temperature increase when concentration of Poloxamer 188 was increased while increasing the concentration of Poloxamer 407, decreased the gelation temperature of the thermosensitive gel [22]. The difference in gelation temperature of each formula was due to differences in hydrophilic and hydrophobic structure of poly(propylene oxide) (PPO) and poly(ethylene oxide) (PEO) from Poloxamer 407 and Poloxamer 188 [23]. The basic structure of poloxamer consists of one PPO block flanked by two PEO blocks with different block lengths and produce various variations of poloxamers [24]. The hydrophobic and hydrophilic ratios of Poloxamer 407 and Poloxamer 188 are 3:7 and 2:8 which makes Poloxamer 407 more hydrophobic. The PPO hydrophobic block would cause a decrease in gelation temperature while the PEO hydrophilic block would increase the gelation temperature. Thus, the greater PPO ratio of the VALS gel formulation, the lower gelation temperature of the preparation [16]. The data obtained showed that the gelation temperature of each formula was significantly different ($p < 0.05$).

3.2.2. Effect of poloxamer on pH, drug content and spreadibility

The pH test was carried out to see whether the pH of the transdermal gel preparation could be well tolerated by the skin. Preparations that have a pH outside this range could potentially cause problems such as irritation, itching or burning of the skin [25]. The result of this study is presented in Table 2. All the measured pH was found to meet the requirement. The pH of preparation than can be tolerated by skin is in the 4-10 range. Statistical analysis showed that the poloxamer concentration increased the pH of the preparation but not significant ($p > 0.05$) [26]. Meanwhile, the results obtained in Table 2 revealed that the drug content in both formulas was found to meet the requirements for drug content standard within 95-105% [27]. Based on these data, it was known that the content of each drug in both formulations was not statistically differrent ($p > 0.05$). Accordingly, it can be seen that the difference in the concentration of poloxamer did not significantly influence the drug content in the preparation and also shows that the gel preparation had uniform drug content that ensure more accurate dosage when given to the patient. Additionally, spreadability of all formulations showed in Tabel 2 indicate that F2 had a higher spreadibility that F3. This was because F3 contained poloxamer with a higher concentration that made the viscosity of the preparation is also higher and the spreadability decreased. Based on these data, it was known that the spreadibility of the VALS gel in the two formulas has a significant difference ($p < 0.05$) due to the difference in the concentration of poloxamer in the two formulas. Spreadibility is one of the most important physical characteristics in a semi-solid preparation because it is responsible for administering the dose to the target and also for the ease of application [28]

Table 2. The result of pH, drug content and spreadibility test of VALS thermosensitive gel (means ± SD., n = 3)

Formula	pH	Drug content (%)	Spreadibility (cm^2)
F2	4.05 ± 0.0	99.19 ± 0.03	7.15 ± 0.2
F3	4.15 ± 0.0	97.11 ± 0.02	5.99 0.2

3.2.3. Effect of poloxamer concentration on viscosity and rheological properties

Viscosity is the most important rheological parameter to indicate the flow of a preparation [29]. The results obtained are depicted in Fig. 3., indicating that the viscosity of F3 was higher than of F2 due to the fact that F3 contained a higher concentration of poloxamer combination when compared to F2. The cross-linking of the

hydrophobic groups of the poloxamer affects the gel strength. The fewer cross-links formed, the softer the gel and the lower the viscosity of the gel [30]. From these data, it was also found that increase in temperature can increasing the viscosity of the preparation, especially when at body temperature which indicates that at cold temperatures and room temperature the preparation was still in liquid form, while when it reached body temperature, the preparation turned into a gel. Importantly, rheology is the study of the flow of a liquid preparation, especially non-Newtonian liquids exhibited by materials such as polymers, a long chain molecule that most often exhibit a pseudoplastic type of flow which shows a decrease in viscosity when the force increases. In the gel rheology test, the following results obtained is showed in Fig. 3. These results indicate that both formulas F2 and F3 had a pseudoplastic flow type because there was a decrease in viscosity along with an increase of spindle speed during measurement. Based on these data, it was observed that poloxamer concentration had a significant effect on the viscosity of the formulas ($p < 0.05$).

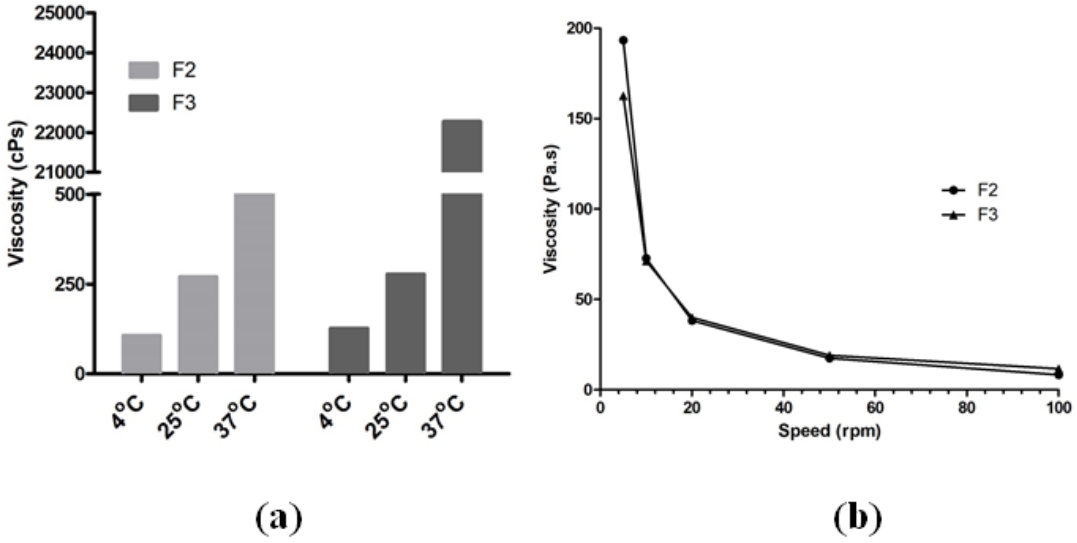


Fig. 3. (a) Viscosity of VALS thermosensitive gel in different temperature; (b) Rheology of VALS thermosensitive gel with variety of of spindle rotation (rpm) (means ± SD., n = 3).

3.3. Effect of poloxamer concentration in *in vitro* release and permeation of VALS

The following graph presented in Fig. 4 shows the amount of drug released from the preparation after 8 hours from F2 was 23.07 ± 0.04 mg and F3 was 28.30 ± 0.1 mg. Moreover, Fig. 4 shows the amount of *in vitro* drug permeated from the preparation after 8 hours. VALS permeated from F2 and F3 were 1.12 ± 0.007 mg 2.01 ± 0.005 mg, respectively. Performed in *ex vivo* study, the amount of VALS permeated from the formulation after 8 were 0.5 ± 0.002 mg and 0.66 ± 0.0005 mg for F2 and F3, respectively. Analyzed statistically, the release and permeation profiles of VALS in *in vitro* and *ex vivo* studies from both formulations were significantly different ($p < 0.05$). The difference on the release profile might be due to the concentration of Poloxamer used. The increasing the concentration of Poloxamer 188 could increase drug release from the preparation [31]. Poloxamer 188 has a relatively shorter chain and a smaller molar ratio or tendency to interact with water. Thus, it could potentially increase the water molecules around the hydrophilic PPO block in Poloxamer 407 which makes the preparation have a tendency to be

eroded and the drug could be more easily released from the preparation. According to the results, F3 was selected for further studies.

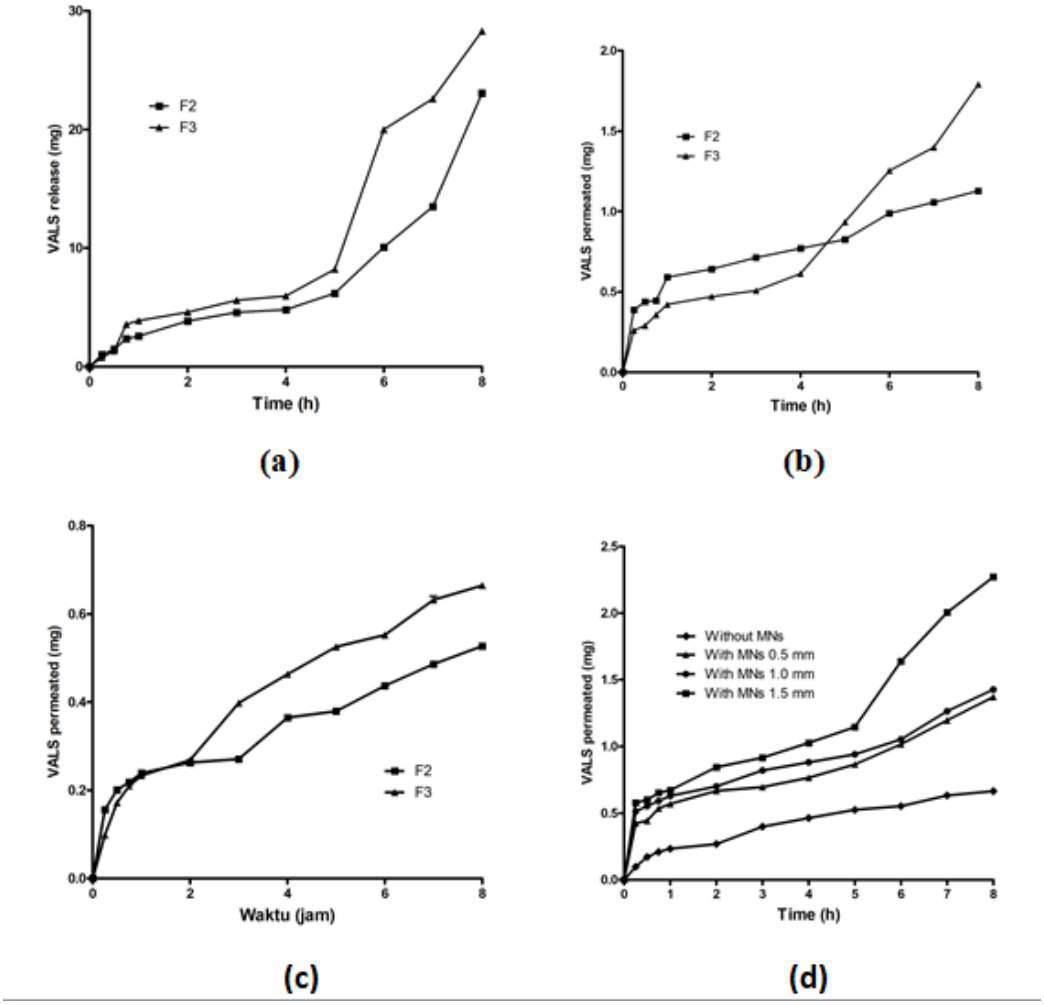


Fig. 4. The result of (a) *in vitro* release of VALS thermosensitive gel; (b) *in vitro* permeation of VALS thermosensitive gel; (c) *ex vivo* permeation of VALS thermosensitive gel; (d) *ex vivo* permeation with and without MNs of F3 (means ± SD., n = 3)

3.4. Effect of MNs in *ex vivo* permeation of VALS

Finally, to improve the transdermal delivery of VALS from thermosensitive hydrogels, solid MNs were used. In the permeation profile investigation using MNs, three types of Dermaroller® with different needle lengths (0.5 mm, 1.0 mm and 1.5 mm) were used to see the effect of MNs needle length on VALS permeation in F3 which showed better physical characteristic, release profile, i*n vitro* permeation and *ex vivo* permeation. Fig. 4 shows the amount of permeated VALS up to 8 hours. In the test without MNs, VALS permeated was found to be only 0.66 ± 0.0005 mg. On the other side, preparations combined with MNs showed an increase in the amount of drug permeated. The combination with 0.5 mm MNs showed the amount of drug permeated of 1.37 ± 0.0054 mg, the combination with 1.0 mm MNs was 1.43 ± 0.0055 mg and the combination with 1.5 mm MNs was 2. 27 ± 0.01 mg. These results showed that the use of MNs could successfully increase the permeation of transdermal preparations because it could form

micro pores on the skin so the drug would easily pass through the SC which is the largest barrier component in the skin. Furthermore, it was observed that the longer the MNs needle, the greater the amount of drug permeated [9]. The results obtained showed that there was a significant increase in the amount of permeated VALS ($p < 0.05$) when VALS was combined with MNs so that it could potentially increase the amount of VALS that entered the body. Overall, the results obtained in this study served as a proof of concept showing that the permeation of VALS through the skin from thermosenstive hydrogel could potentially be improved using solid MNs. This could be beneficial in the treatment of hypertension. However, *in vivo* animal works should now be carried out.

4. Conclusion

This study investigated the potency of a transdermal delivery system to enhance the VALS permeated through the skin layer using poloxamer-based thermosensitive gel. According on the findings presented in this study, it was shown that VALS thermosensitive gel was successfully prepared using combination of poloxamer 188 and poloxamer 407, where concentration of poloxamer affected the physical properties of thermosensitive gel. Furthermore, the combination of VALS transdermal thermosensitive gel and solid MNs enhanced the permeation of VALS in *ex vivo* rat skin. The major advancement of the combinatorial delivery system we have presented in this work, led to higher permeation through the skin that could improve the efficiency of hypertension therapy, hypothetically. Building from these stated results, further studies including *in vivo* efficacy studies should be carried out to completely investigate the therapeutic efficacy of this approach in hypertension animal models.

AUTHORS CONTRIBUTION

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

References

1. Dipiro JT, Yee GC, Posey LM, Haines ST, Nolin TD, Ellingrod V. Pharmacotherapy: A Pathophysiologic Approach. Eleventh E. New York: The McGraw-Hill Companies, Inc.; 2020.

2. Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M. Enhanced transdermal delivery of an anti-hypertensive agent via nanoethosomes: Statistical optimization, characterization and pharmacokinetic assessment. Int J Pharm. Elsevier B.V.; 2013;443:26–38.

3. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. Nat Rev Nephrol. Springer US; 2020;16:223–37.

4. Zhou B, Carrillo-Larco RM, Danaei G, Riley LM, Paciorek CJ, Stevens GA, et al. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet. 2021;398:957–80.

5. Bhosale SS, Avachat AM. Design and development of ethosomal transdermal drug delivery system of valsartan with preclinical assessment in Wistar albino rats. J Liposome Res. 2013;23:119–25.

6. Ahad A, Aqil M, Ali A. Investigation of antihypertensive activity of carbopol valsartan transdermal gel containing 1,8-cineole. Int J Biol Macromol. Elsevier B.V.; 2014;64:144–9.

7. Khan S, Minhas MU, Tekko IA, Donnelly RF, Thakur RRS. Evaluation of microneedles-assisted in situ depot forming poloxamer gels for sustained transdermal drug delivery. Drug Deliv Transl Res. Drug Delivery and Translational Research; 2019;9:764–82.

8. Ahmed KS, Shan X, Mao J, Qiu L, Chen J. Derma roller® microneedles-mediated transdermal delivery of doxorubicin and celecoxib co-loaded liposomes for enhancing the anticancer effect. Mater Sci Eng C [Internet]. Elsevier; 2019;99:1448–58. Available from: https://doi.org/10.1016/j.msec.2019.02.095

9. Tobin K V., Fiegel J, Brogden NK. Thermosensitive gels used to improve microneedle-assisted transdermal delivery of naltrexone. Polymers (Basel). 2021;13:1–15.

10. Chen J, Zhou R, Li L, Li B, Zhang X, Su J. Mechanical, rheological and release behaviors of a poloxamer 407/poloxamer 188/carbopol 940 thermosensitive composite hydrogel. Molecules. 2013;18:12415–25.

11. Permana AD, Utomo E, Pratama MR, Amir MN, Anjani QK, Mardikasari SA, et al. 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. 2021;13:18128–41.

12. Enggi CK, Isa HT, Sulistiawati S, Ardika KAR, Wijaya S, Asri RM, et al. 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 [Internet]. Elsevier B.V.; 2021;609:121182. Available from: https://doi.org/10.1016/j.ijpharm.2021.121182

13. Nief RA, Hussein AA. Preparation and Evaluation of Meloxicam Microsponges as Transdermal Delivery System. 2014;23.

14. Verma S, Chaudhary B. Preparation and Evaluation of Novel In Situ Gels Containing Acyclovir for the Treatment of Oral Herpes Simplex Virus Infections Simultaneous Estimation of Mycophenolate Mofetil and Prednisolone View project Preparation and Evaluation of Novel In Situ Gels. Artic Sci World J. 2014;2014.

15. Khattab A, Marzok S, Ibrahim M. Development of optimized mucoadhesive thermosensitive pluronic based in situ gel for controlled delivery of Latanoprost: Antiglaucoma efficacy and stability approaches. J Drug Deliv Sci Technol. Elsevier; 2019;53:101134.

16. Fathalla ZMA, Vangala A, Longman M, Khaled KA, Hussein AK, El-Garhy OH, et al. Poloxamer-based thermoresponsive ketorolac tromethamine in situ gel preparations: Design, characterisation, toxicity and

transcorneal permeation studies. Eur J Pharm Biopharm. Elsevier B.V.; 2017;114:119–34.

17. Putri HE, Utami RN, Aliyah, Wahyudin E, Oktaviani WW, Mudjahid M, et al. Dissolving Microneedle Formulation of Ceftriaxone: Effect of Polymer Concentrations on Characterisation and Ex Vivo Permeation Study. J Pharm Innov [Internet]. Springer US; 2021; Available from: https://doi.org/10.1007/s12247-021-09593-y

18. Ananda PWR, Elim D, Zaman HS, Muslimin W, Tunggeng MGR, Permana AD. Combination of transdermal patches and solid microneedles for improved transdermal delivery of primaquine. Int J Pharm [Internet]. Elsevier B.V.; 2021;609:121204. Available from: https://doi.org/10.1016/j.ijpharm.2021.121204

19. Ramadon D, Permana AD, Courtenay AJ, McCrudden MTC, Tekko IA, McAlister E, et al. Development, Evaluation, and Pharmacokinetic Assessment of Polymeric Microarray Patches for Transdermal Delivery of Vancomycin Hydrochloride. Mol Pharm. 2020;17:3353–68.

20. Jain D. Newer Trends in In Situ Gelling Systems for Controlled Ocular Drug Delivery. J Anal Pharm Res. 2016;2:1–16.

21. Mura S, Manconi M, Sinico C, Valenti D, Maria A. Penetration enhancer-containing vesicles (PEVs) as carriers for cutaneous delivery of minoxidil. 2009;380:72–9.

22. Zhang K, Shi X, Lin X, Yao C, Shen L, Feng Y. Poloxamer-based in situ hydrogels for controlled delivery of hydrophilic macromolecules after intramuscular injection in rats. Drug Deliv. 2015;22:375–82.

23. Permana AD, Nurul R, Layadi P, Himawan A, Juniarti N, Kurnia Q, et al. Thermosensitive and mucoadhesive in situ ocular gel for effective local delivery and antifungal activity of itraconazole nanocrystal in the treatment of fungal keratitis. Int J Pharm. Elsevier B.V.; 2021;602:120623.

24. Li X, Hyun K. Rheological study of the effect of polyethylene oxide (PEO) homopolymer on the gelation of PEO-PPO-PEO triblock copolymer in aqueous solution. Korea Aust Rheol J. 2018;30:109–25.

25. Alberti I, Grenier A, Kraus H, Carrara DN. Pharmaceutical development and clinical effectiveness of a novel gel technology for transdermal drug delivery. Expert Opin Drug Deliv. 2005;2:935–50.

26. Jarmolowska KM. THE EFFECT OF POLOXAMER 407 ON THE PROPERTIES OF HYDROPHILIC GELS CONTAINING LACTIC ACID COMPLEXED WITH CHITOSAN. Prog Chem Appl Chitin Its. 2010;XV:143–8.

27. Lalitha N, Sanjay PPN, Vyshak MG, Kadri U, Pharmacy AC, Lalbagh N, et al. Stability-Indicating Reverse Phase HPLC Method for the Determination of Cefazolin. 2010;9:45–50.

28. Rençber S, Karavana SY, Şenyiğit ZA, Eraç B, Limoncu MH, Baloğlu E. Mucoadhesive in situ Gel Formulation for Vaginal Delivery of Clotrimazole: Formulation, Preparation, and in vitro/in vivo Evaluation. Pharm Dev Technol. 2017;22:551–61.

29. Aho J, Boetker JP, Baldursdottir S, Rantanen J. Rheology as a tool for evaluation of melt processability of innovative dosage forms. Int J Pharm. Elsevier B.V.; 2015;494:623–42.

30. Rarokar NR, Saoji SD, Khedekar P. Investigation of Effect of Extensively Used Polymers on Thermoreversible Properties of Pluronic ® Tri-Block Polymer. 2017;5:29–39.

31. Inal O, Yapar EA. Effect of mechanical properties on the release of meloxicam from poloxamer gel bases. Indian J Pharm Sci. 2013;75:700–6.

BUKTI ACCEPTED

Universitas Hasanuddin

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

Your Submission #AAPSPT-D-22-00521R2 Has Been Accepted for Publication

1 message

AAPSPT Editorial Office <em@editorialmanager.com> Wed, Nov 9, 2022 at 5:20 AM
Reply-To: AAPSPT Editorial Office <aapsptsubmit@aaps.org>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

CC: cbwu2000@yahoo.com, "Nirmayanti Nirmayanti" nirmayanti18n@student.unhas.ac.id, "Alhidayah Alhidayah" alhidayah18n@student.unhas.ac.id, "Jessica Theodor Usman" usmanjt18n@student.unhas.ac.id, "Julika Fajrika Nur" nurjf18n@student.unhas.ac.id, "Muh. Nur Amir" muhnuramir@unhas.ac.id

Re: Manuscript AAPSPT-D-22-00521R2
Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An in vivo proof of concept study
AAPS PharmSciTech

Dear Dr. Permana,

I am pleased to inform you that your manuscript "Combinatorial approach of thermosensitive hydrogels and solid microneedles to improve transdermal delivery of valsartan: An in vivo proof of concept study" has been accepted for publication in AAPS PharmSciTech.

It was accepted on 11/08/2022.

IMPORTANT

COVID 19 and IMPACT OF PUBLICATION
As a result of the significant disruption that is being caused by the COVID-19 pandemic, we are experiencing production delays. You will receive your proof as soon as it is made available. Thank you for your patience and understanding.

*NOTE: Transfer of copyright will be processed by our publisher, Springer Nature. Once your article has been completely typeset, you will receive a questionnaire. Once the questionnaire is complete, a proof will be released. If you do not receive an email within 2 weeks from acceptance, please contact Matt Baughman at BaughmanM@aaps.org.

Once your manuscript enters the production queue and the copyediting process is completed, the Corresponding Author will receive a link to the online proof via email. This proof version could contain queries raised by the Copyeditor and you will need to address these in the proof review stage. To ensure the efficiency of the publication process, you will be asked to respond to the galley proof within 48 hours. Instructions for the proof stage will be sent in the email message containing the link to the online proof.

The contents of your accepted manuscript are confidential and are not to be copyrighted, discussed or published elsewhere in any language (including the Internet).

Thank you for submitting your work to AAPS PharmSciTech.

Robert O. Williams III, Ph.D.
Editor-in-Chief
AAPS PharmSciTech

*NOTE FROM SPRINGER NATURE (PUBLISHER)
Please note that this journal is a Transformative Journal (TJ). Authors may publish their research with us through the traditional subscription access route or make their paper immediately open access through payment of an article-processing charge (APC). Authors will not be required to make a final decision about access to their article until it has been accepted.

Authors may need to take specific actions to achieve compliance with funder and institutional open access mandates. If your research is supported by a funder that requires immediate open access (e.g. according to Plan S principles) then you should select the gold OA route, and we will direct you to the compliant route where possible. For authors selecting the subscription publication route our standard licensing terms will need to be accepted, including our self-archiving policies. Those standard licensing terms will supersede any other terms that the author or any third party may assert apply to any version of the manuscript.

Find out more about compliance:
https://www.springernature.com/gp/open-research/funding/policy-compliance-faqs

---