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

Submission Confirmation for Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation

1 message

Journal of Biomaterials Science Polymer Edition <em@editorialmanager.com>

Wed, Jul 13, 2022 at 5:16 PM

Reply-To: Journal of Biomaterials Science Polymer Edition <coopers@chbmeng.ohio-state.edu>

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

Dear Dr. Permana,

Your submission entitled "Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation" has been received by journal Journal of Biomaterials Science: Polymer Edition

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is <https://www.editorialmanager.com/jbs/>.

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Thank you for submitting your work to this journal.

Kind regards,

Journal of Biomaterials Science: Polymer Edition

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

**BUKTI
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REVIEWERS**



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

(Journal of Biomaterials Science: Polymer Edition) A revise decision has been made on your submission1 message

Stuart Cooper <em@editorialmanager.com>

Sat, Aug 13, 2022 at 1:30 AM

Reply-To: Stuart Cooper <coopers@chbmeng.ohio-state.edu>

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

Ref.: Ms. No. JBS-D-22-00297

Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation
Journal of Biomaterials Science: Polymer Edition

Dear Dr. Permana,

Your manuscript has been reviewed by two referees who suggest some significant improvements are required before the manuscript is suitable for publication.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Your revision is due by Sep 11, 2022.

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

Your revision is due by Sep 11, 2022.

To submit a revision, go to <https://www.editorialmanager.com/jbs/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Stuart Cooper
Editor-in-Chief
Journal of Biomaterials Science: Polymer Edition

Comments from the Editors and Reviewers:

Reviewer #2:

The manuscript is very well written with organized experimentation section. It touches all aspects of formulation and analytical testing. However, I would recommend major additions which can further add to quality of manuscript.

Section 2.2 – What was the function of DMDM hydantoin in formulation and how did it add to the value of the transdermal gel?

Section 2.4 – Please discuss what speed and spindle were used to measure viscosity. Additionally, viscosity is also impacted by the temperature, what was the temperature of measurement?

Section 2.5 – what was the diameter of glass plate?

Section 2.8 – How was drug content calculated – against a standard or calibration curve? Please add this detail.

Section 2.9 – Please add the pore size of membrane. Please add dimensions of the membrane.

“one gram of valsartan gel was then dispersed on the cellophane membrane.” Please specify dispersed – was it spread?

Please provide a figure of the experimental set up – will help the text.

How many number of replicates were performed?

Section 2.11 – Please add a figure of Franz diffusion cell setup

Can you please specify the details of skin – thickness, hairless, full thickness, how many pores were made from Dermaroller on the skin?

Was any integrity testing performed on the skin after use of Dermaroller?

What was the surface area of diffusion?

What was the quantity of gel on the donor membrane?

Section 3.2 – please add description on why pH was adjusted to 7.4 when the same paragraph mentions pH of skin is 4.1 – 5.8.

Section 3.3 – Paragraph mentions that neutralization increases the viscosity. The pH of F1 was recorded at 7.41 while pH of F4 is at 7.56. Table 1 shows pH of formulation from F1-F2-F3-F4 increases – could this be the cause of high viscosity rather than increase in carbomer content – discussion on this would be helpful.

Section 3.7 – according to the experimental setup “one gram of valsartan gel was then dispersed on the cellophane membrane” which is equivalent to 10mg of valsartan. F1 has more 10mg of drug release. Please clarify.

Figure 2A shows 80% of valsartan was released in first two hours followed by remaining 20% in the next 6 hours. A clarification of this results will be helpful.

Section 3.8 – please also plot the occlusive data for control. Section 2.10 experimental section states that occlusive study was also performed on control. Also discuss how does the samples F1 through F4 compare against the control.

Section 3.9 – This section needs to be updated significantly. Data only for F1 is discussed. Please discuss in detail about comparison of each of the following:

For control, 0.5mm, 1.0mm and 1.5mm – F1 vs F2 vs F3 vs F4

For each formulation compare control vs 0.5mm vs 1.0mm vs 1.5mm.

A figure of dermaroller perforated skin will be helpful

Also, more acceptable way of plotting data is plot of “average cumulative percent drug release vs time”. This will also give release profile for each formulation.

Reviewer #4: 1. Thorough language revision and grammar check are needed.

2. Mention the abbreviation consistently throughout the manuscript. In the abstract, it is mentioned 'VALS,' but in the main content, it is mentioned 'VAL & VALS,' and it is also mentioned 'valsartan' in the main text.

3. It is mentioned that 'Higher amount of VALS released were observed as the concentration of Carbopol increase in

the formulation.' Please explain this statement. Because in the same paragraph, it is noticed that the drug release is decreased due to the (8.44±0.25 mg for F4) increased concentration of the carbopol.

4. It is mentioned that the 'Higher occlusivity could increase skin hydration which facilitate drug penetration.' But it could take longer for the skin to reverse to its normal condition. So, it may irreversibly damage the skin. Please explain.

5. Occlusivity also influence the drug permeation. Is that considered in this study?

6. Please follow the journal template, including the subsections' numbering.

7. Include permeation coefficient in the results.

8. Describe the rationale and characteristics of the optimized formulation in detail.

9. In vitro hemolysis- is done only for the optimized formulation or all formulations?

10. No skin irritation-requires strong justification, not just only pH.

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. ([Remove my information/details](#)). Please contact the publication office if you have any questions.

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Journal of Biomaterials Science: Polymer Edition

Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation --Manuscript Draft--

Manuscript Number:	JBS-D-22-00297R1
Full Title:	Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation
Short Title:	Valsartan hydrogel with solid microneedles
Article Type:	Original Research Paper
Keywords:	Valsartan; polymeric hydrogel; transdermal delivery; solid microneedles
Corresponding Author:	Andi Dian Permana, Ph.D INDONESIA
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First Author:	Cindy Kristina Enggi
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Order of Authors:	Cindy Kristina Enggi Mega Tri Satria Nirmayanti Nirmayanti Jessica Theodor Usman Julika Fajrika Nur Rangga Meidianto Asri Nana Juniarti Natsir Djide Andi Dian Permana, Ph.D
Order of Authors Secondary Information:	
Manuscript Region of Origin:	INDONESIA
Abstract:	Valsartan (VAL) is used as a first-line agent to treat hypertension. However, VAL exhibits poor absorption and low bioavailability when administrated orally. To overcome these issues, VAL transdermal gel was developed in this study, where Carbopol was used as the gel matrice. Additionally, solid microneedles (Dermaroller®) with various needle lengths were combined with transdermal gel to improve its permeation across the stratum corneum as a skin barrier. Developed formulations were further evaluated for various parameters, including pH, viscosity, spreadability, extrudability, gel strength, drug content, ex vivo permeation, in vitro release, occlusivity, and hemolysis. The results showed that all formulations exhibited desired physical characteristics without any potential to cause toxicity. Moreover, this approach showed that using microneedles could significantly enhance the permeation of VAL up to 3 folds compared to untreated skin. The use of microneedles 1.5 mm was found to be the optimum combination to improve VA permeation without affecting skin integrity. As much as 1.69 ± 0.004 mg of VAL permeated after 8 h. Finally, it could be concluded that this work had successfully developed a new approach for VALS drug delivery and could potentially show a significant impact on the treatment of hypertension. Further in

	vivo work should be considered.
Response to Reviewers:	Ref.: Ms. No. JBS-D-22-00297 Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation Journal of Biomaterials Science: Polymer Edition Response to Reviewers We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. We have made some changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each of the reviewers' comments in detail below. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript. Reviewer #2: The manuscript is very well written with organized experimentation section. It touches all aspects of formulation and analytical testing. However, I would recommend major additions which can further add to quality of manuscript. Response to Reviewer We are very thankful to the Reviewer for taking the time to review this manuscript and for the expert review, providing helpful comments. We are glad that the Reviewer thinks that our work is well written and organized. We have made a number of key changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each one of the reviewers' comments in detail. Section 2.2 – What was the function of DMDM hydantoin in formulation and how did it add to the value of the transdermal gel? Response: We thank the reviewer for the question. DMDM hydantoin was used a preservative. As it is in the liquid form, it was added into the gel base with the valsartan solution, as explained in the revised manuscript. Section 2.4 – Please discuss what speed and spindle were used to measure viscosity. Additionally, viscosity is also impacted by the temperature, what was the temperature of measurement? Response: We thank the reviewer for the suggestion. As a result, we have added the explanation in the revised manuscript. The evaluation was performed at room temperature at 50 rpm using 50 g of sample, spindle number 7 and a total time of 60 s for one measurement. Section 2.5 – what was the diameter of glass plate? Response: We thank the reviewer for the question. The diameter of glass plate was 25 cm. We have added the explanation in the revised manuscript. Section 2.8 – How was drug content calculated – against a standard or calibration curve? Please add this detail. Response: We thank the reviewer for the question. The determination of the concentration of VAL was performed by plotting the absorbance obtained to the calibration curve made from the standard solution of VAL in the concentration ranging from 0.5 µg/mL to 32 µg/mL. We have added the explanation in the revised manuscript. Section 2.9 – Please add the pore size of membrane. Please add dimensions of the membrane. Response: We thank the reviewer for the suggestion. The in vitro release profile of valsartan gel was measured using the membrane dialysis method (Permana et al., 2020a, 2019). Spectra-Por®, pore size of 12,000–14,000 MWCO dialysis membrane (Spectrum Medical Industries, CA, USA) was prepared and immersed in PBS solution for 24 hours. One gram of valsartan gel was accurately weighed and placed in the dialysis membrane (25 x 50 mm) using spatula. We have included these in the revised manuscript. “one gram of valsartan gel was then dispersed on the cellophane membrane.” Please specify dispersed – was it spread? Response: We thank the reviewer for the suggestion. One gram of valsartan gel was accurately

weighed and placed in the dialysis membrane. We have included this in the revised manuscript.

Please provide a figure of the experimental set up – will help the text.

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

How many number of replicates were performed?

Response:

We thank the reviewer for the suggestion. The experiment was carried out in triplicates. We have now included this in the revised manuscript.

Section 2.11 – Please add a figure of Franz diffusion cell setup

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

Can you please specify the details of skin – thickness, hairless, full thickness, how many pores were made from Dermaroller on the skin?

Response:

We thank the reviewer for the suggestion. The ex vivo permeation study was conducted using vertical Franz cell and abdominal skin from Wistar rats. In order to obtain the skin, the rat's hair was removed using an electrical shaver and hair removal cream. Following this, the skins were collected after the rats were sacrificed. The full-thickness skins were collected by removing the fats attached to the skins. The skin was washed using PBS prior to the experiment. The diameters of the skins were 2.65 ± 0.18 mm. The skin collection was ethically permitted by the Health Ethical Committee at the Faculty of Medicine, Hasanuddin University, Indonesia. We have now included these in the revised manuscript.

Was any integrity testing performed on the skin after use of Dermaroller?

Response:

We thank the reviewer for the suggestion. We have performed the skin integrity evaluation and have included the results in the revised manuscript.

What was the surface area of diffusion?

Response:

We thank the reviewer for the question. The diffusion area was 4.9 cm². We have now included this in the revised manuscript.

What was the quantity of gel on the donor membrane?

Response:

We thank the reviewer for the suggestion. In this study, 1 g of gel formulation (equal to 10 mg of VAL) was applied to the skin. We have now included this in the revised manuscript.

Section 3.2 – please add description on why pH was adjusted to 7.4 when the same paragraph mentions pH of skin is 4.1 – 5.8.

Response:

We thank the reviewer for the suggestion. We have rephrased the explanations, as follows:

The pH of healthy skin is reported to be around 4.1 – 5.8 (Proksch, 2018). pH value plays an important role in maintaining skin barrier function. Acidic pH of the skin is responsible for controlling the presence of skin microflora (Lambers et al., 2006; Paudel et al., 2010). The barrier integrity of the skin was also affected by the pH value (Gorzalanny et al., 2020). In this study, we used Carbomer as the main gelling agent. It has been reported by numerous studies that the gel formation prepared by Carbomer could occur effectively at neutral pH (around 7) (Tripathi et al., 2018; Zhang et al., 2018). Therefore, the addition of TEA could neutralize the pH of the gels. The result showed that pH values of VAL transdermal gel were between 7.41 ± 0.01 and 7.56 ± 0.01 (Table 2). Although the obtained results were not in the range of the normal skin pH, a study conducted by Alberti et al., 2005 stated that transdermal formulations with pH values of 4-10 are still well tolerated. In line with this study, a previous study showed that a pH value of 7 did not result in skin irritation (Al-Suwayeh et al., 2014). Accordingly, the developed VAL transdermal gels were considered safe and could not possibly cause irritation to the skin.

We have now included this in the revised manuscript.

Section 3.3 – Paragraph mentions that neutralization increases the viscosity. The pH of F1 was recorded at 7.41 while pH of F4 is at 7.56. Table 1 shows pH of formulation from F1-F2-F3-F4 increases – could this be the cause of high viscosity rather than increase in carbomer content – discussion on this would be helpful.

Response:
We thank the reviewer for the suggestion. As a result, we have included the explanations, as follows:
Additionally, it was also important to note that the pH of the formulations also increased with the increase of Carbomer and TEA concentration. As previously explained, the neutralization could affect the viscosity of the gels. The pH of F4 was found to be highest, followed by F3, F2 and F1. This could result in the higher viscosity of the formulation following the pH values of each formulation.
We have now included this in the revised manuscript.
Section 3.7 – according to the experimental setup “one gram of valsartan gel was then dispersed on the cellophane membrane” which is equivalent to 10mg of valsartan. F1 has more 10mg of drug release. Please clarify.

Response:
We thank the reviewer for pointing this out. In this study, we have calculated the drug recovery, showing the percentage more than 100%. However, this still met the requirement of drug content recovery set by the International Council for Harmonization (ICH), which was in the range of 95-105%. (Walfish, 2006). Accordingly, this could be the reason that the release amount was more than 10 mg.
Figure 2A shows 80% of valsartan was released in first two hours followed by remaining 20% in the next 6 hours. A clarification of this results will be helpful.

Response:
We thank the reviewer for the suggestion. As a result, we have included the explanations, as follows:
Specifically, it was found that in the first 2 hours, all formulations exhibited burst release, showing the release percentage of around 80%. Afterwards, the sustained release manner was shown in the following hours. The burst release occurred possibly due to the presence of VAL on the outer surface of the gel or in the gel surface layers voids.
We have now included this in the revised manuscript.
Section 3.8 – please also plot the occlusive data for control. Section 2.10 experimental section states that occlusive study was also performed on control. Also discuss how does the samples F1 through F4 compare against the control.
We thank the reviewer for the suggestion. As a result, we have included the results of the control and these explanations, as follows:
Additionally, it was also observed that there was no significant difference ($p > 0.05$) in the occlusivity values between all formulations with the control formulations,
We have now included this in the revised manuscript.
Section 3.9 – This section needs to be updated significantly. Data only for F1 is discussed. Please discuss in detail about comparison of each of the following:
For control, 0.5mm, 1.0mm and 1.5mm – F1 vs F2 vs F3 vs F4

Response:
We thank the reviewer for the suggestion. We have now included this in the revised manuscript.
For each formulation compare control vs 0.5mm vs 1.0mm vs 1.5mm.

Response:
We thank the reviewer for the suggestion. We have now included this in the revised manuscript.
A figure of dermaroller perforated skin will be helpful

Response:
We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

Also, more acceptable way of plotting data is plot of “average cumulative percent drug release vs time”. This will also give release profile for each formulation.

Response:
We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

Reviewer #4: 1. Thorough language revision and grammar check are needed.

Response:
We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. We have re-read the manuscript and have made a great effort to improve the English throughout.

2. Mention the abbreviation consistently throughout the manuscript. In the abstract, it is

mentioned 'VALS,' but in the main content, it is mentioned 'VAL & VALS,' and it is also mentioned 'valsartan' in the main text.

Response:

We thank the reviewer for the suggestion. We have mentioned the abbreviation consistently in the revised manuscript.

3. It is mentioned that 'Higher amount of VALS released were observed as the concentration of Carbopol increase in the formulation.' Please explain this statement. Because in the same paragraph, it is noticed that the drug release is decreased due to the (8.44±0.25 mg for F4) increased concentration of the carbopol.

Response:

We thank the reviewer for pointing this out. We do apologize for the confusion. Indeed, Carbopol concentration affected the release of VAL. A higher amount of VAL released was observed as the concentration of Carbopol decreased in the formulation. We have clarified this in the revised manuscript.

4. It is mentioned that the 'Higher occlusivity could increase skin hydration which facilitate drug penetration.' But it could take longer for the skin to reverse to its normal condition. So, it may irreversibly damage the skin. Please explain.

Response:

We thank the reviewer for the suggestion. As a result, we have included the explanations, as follows:

The occlusivity of formulations would affect the amount of drugs in the skin. Higher occlusivity could increase skin hydration which facilitates drug penetration. This is due to the reduction of corneocyte gaps (Ammar et al., 2016; Permana et al., 2020b).

Additionally, it was also observed that there was no significant difference ($p > 0.05$) in the occlusivity values between all formulations with the control formulations.

Accordingly, the addition of VAL did not alter the occlusivity of Carbomer-based gels.

Several studies have shown the benefits of the occlusivity of topical preparation in the transdermal delivery system. The occlusivity of the preparations did not cause damage and irritation to the skin (Shrotriya et al., 2018, 2017; Wavikar and Vavia, 2013).

Importantly, the values obtained in our study were found to be comparable with several previous reports. Accordingly, it might be hypothesized that the occlusivity properties of the gel did not possibly cause any damage in the skin.

We have now included this in the revised manuscript.

5. Occlusivity also influence the drug permeation. Is that considered in this study?

Response:

We thank the reviewer for the question. In this study, we found that there was no significant difference in the occlusivity in all formulations. Therefore, the viscosity and the combination with Dermalrollers® were considered as the main reason for the difference in the drug permeation profile.

6. Please follow the journal template, including the subsections' numbering.

Response:

We thank the reviewer for the suggestion. We have used the template of the journal and followed the guidelines required.

7. Include permeation coefficient in the results.

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

8. Describe the rationale and characteristics of the optimized formulation in detail.

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

9. In vitro hemolysis- is done only for the optimized formulation or all formulations?

Response:

We thank the reviewer for the question. In this study, we evaluated the hemolysis ability of VAL from F1 as the optimum formulation. We have now included this in the revised manuscript.

10. No skin irritation-requires strong justification, not just only pH.

Response:

We thank the reviewer for the suggestion. Indeed, the skin irritation study is requires, Accordingly, we have rephrased to explanation to avoid the confusion. The developed VAL transdermal gels were considered safe and could not possibly cause irritation to

the skin. To show the efficacy of this combination, further in vivo studies should be considered to assess the pharmacokinetic, pharmacodynamic and skin irritation studies.

REFERENCES:

- Al-Suwayeh, S.A., Taha, E.I., Al-Qahtani, F.M., Ahmed, M.O., Badran, M.M., 2014. Evaluation of skin permeation and analgesic activity effects of carbopol lornoxicam topical gels containing penetration enhancer. *Sci. World J.* 2014. <https://doi.org/10.1155/2014/127495>
- Alberti, I., Grenier, A., Kraus, H., Carrara, D.N., 2005. Pharmaceutical development and clinical effectiveness of a novel gel technology for transdermal drug delivery. *Expert Opin. Drug Deliv.* 2, 935–950. <https://doi.org/10.1517/17425247.2.5.935>
- Ammar, H.O., Ghorab, M.M., Mostafa, D.M., Ibrahim, E.S., 2016. Folic acid loaded lipid nanocarriers with promoted skin antiaging and antioxidant efficacy. *J. Drug Deliv. Sci. Technol.* 31, 72–82.
- Gorzelanny, C., Mess, C., Schneider, S.W., Huck, V., Brandner, J.M., 2020. Skin barriers in dermal drug delivery: Which barriers have to be overcome and how can we measure them? *Pharmaceutics* 12, 1–31. <https://doi.org/10.3390/pharmaceutics12070684>
- Lambers, H., Piessens, S., Bloem, A., Pronk, H., Finkel, P., 2006. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *Int. J. Cosmet. Sci.* 28, 359–370. <https://doi.org/10.1111/j.1467-2494.2006.00344.x>
- Paudel, K.S., Milewski, M., Swadley, C.L., Brogden, N.K., Ghosh, P., Stinchcomb, A.L., 2010. Challenges and opportunities in dermal/transdermal delivery. *Ther. Deliv.* 1, 109–131. <https://doi.org/10.4155/tde.10.16>
- Permana, A.D., McCrudden, M.T.C., Donnelly, R.F., 2019. Enhanced intradermal delivery of nanosuspensions of antifilaria drugs using dissolving microneedles: A proof of concept study. *Pharmaceutics* 11, 346.
- Permana, A.D., Paredes, A.J., Volpe-Zanutto, F., Anjani, Q.K., Utomo, E., Donnelly, R.F., 2020a. Dissolving microneedle-mediated dermal delivery of itraconazole nanocrystals for improved treatment of cutaneous candidiasis. *Eur. J. Pharm. Biopharm.* 154, 50–61. <https://doi.org/10.1016/j.ejpb.2020.06.025>
- Permana, A.D., Utami, R.N., Courtenay, A.J., Manggau, M.A., Donnelly, R.F., Rahman, L., 2020b. 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.
- Proksch, E., 2018. pH in nature, humans and skin. *J. Dermatol.* 45, 1044–1052. <https://doi.org/10.1111/1346-8138.14489>
- Shrotriya, S., Ranpise, N., Satpute, P., Vidhate, B., 2018. Skin targeting of curcumin solid lipid nanoparticles-engrossed topical gel for the treatment of pigmentation and irritant contact dermatitis. *Artif. Cells, Nanomedicine Biotechnol.* 46, 1471–1482. <https://doi.org/10.1080/21691401.2017.1373659>
- Shrotriya, S.N., Ranpise, N.S., Vidhate, B. V., 2017. Skin targeting of resveratrol utilizing solid lipid nanoparticle-engrossed gel for chemically induced irritant contact dermatitis. *Drug Deliv. Transl. Res.* 7, 37–52. <https://doi.org/10.1007/s13346-016-0350-7>
- Tripathi, P., Kumar, A., Jain, P.K., Patel, J.R., 2018. Carbomer gel bearing methotrexate loaded lipid nanocontainers shows improved topical delivery intended for effective management of psoriasis. *Int. J. Biol. Macromol.* 120, 1322–1334. <https://doi.org/10.1016/j.ijbiomac.2018.08.136>
- Walfish, S., 2006. A statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. *BioPharm Int.* 19, 28–36.
- Wavikar, P., Vavia, P., 2013. Nanolipidgel for enhanced skin deposition and improved antifungal activity. *AAPS PharmSciTech* 14, 222–233. <https://doi.org/10.1208/s12249-012-9908-y>
- Zhang, Y., Ng, W., Hu, J., Mussa, S.S., Ge, Y., Xu, H., 2018. Formulation and in vitro stability evaluation of ethosomal carbomer hydrogel for transdermal vaccine delivery. *Colloids Surfaces B Biointerfaces* 163, 184–191. <https://doi.org/10.1016/j.colsurfb.2017.12.031>

Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation

Cindy Kristina Enggi, Mega Tri Satria, Nirmayanti, Jesscia Theodor Usman, Julika Fajrika Nur, **Rangga Meidianto Asri**, Nana Juniarti Natsir Djide, Andi Dian Permana*

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Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An *ex vivo* proof of concept investigation

Valsartan (VAL) is used as a first-line agent to treat hypertension. However, VAL exhibits poor absorption and low bioavailability when administered orally. To overcome these issues, VAL transdermal gel was developed in this study, where Carbopol was used as the gel matrix. Additionally, solid microneedles (Dermaroller®) with various needle lengths were combined with transdermal gel to improve its permeation across the stratum corneum as a skin barrier.

Developed formulations were further evaluated for various parameters, including pH, viscosity, spreadability, extrudability, gel strength, drug content, *ex vivo* permeation, in vitro release, occlusivity, and hemolysis. The results showed that all formulations exhibited desired physical characteristics without any potential to cause toxicity. Moreover, this approach showed that using microneedles could significantly enhance the permeation of VAL up to 3 folds compared to untreated skin. The use of microneedles 1.5 mm was found to be the optimum combination to improve VAL permeation without affecting skin integrity. As much as 1.69 ± 0.004 mg of VAL permeated after 8 h. Finally, it could be concluded that this work had successfully developed a new approach for VALS drug delivery and could potentially show a significant impact on the treatment of hypertension. Further *in vivo* work should be considered.

Keywords: Valsartan; polymeric hydrogel; transdermal delivery; solid microneedles

1. Introduction

Hypertension is a condition characterized by high blood pressure (BP) in the systemic arteries, expressed by the ratio of systolic and diastolic BP [1]. Hypertension is defined as having systolic BP 140 mmHg or higher and/or having diastolic BP 90 mmHg or higher [2]. It is a major global health problem due to its high prevalence. Around 12.8% (7.5 million) of the total of all annual deaths worldwide were caused by high blood pressure, and it is predicted to increase to 1.56 billion adults with hypertension in 2025

[3]. It is also a significant risk factor for cardiovascular disease (CVD) and chronic kidney disease (CKD) and is the single leading contributor to all-cause death and disability worldwide [4].

Valsartan (VAL) is an antihypertensive drug which belongs to Angiotensin II Receptor Blocker (ARB). The use of VAL does not associate with persistent cough/angioedema due to its ability to not inhibit the breakdown of bradykinin. According to Nixon et al., 2009, among other ARB drugs, VAL is found to be more effective at lowering BP and shows comparable efficacy in patients. However, when administered orally, VAL exhibits low bioavailability (around 10-35%) due to poor absorption in the gastrointestinal tract. Furthermore, food intake is known to affect the pharmacokinetics of VAL by reducing its C_{max} and AUC [6,7]. Therefore, finding an alternative route for delivering valsartan is needed.

Transdermal drug delivery system (TDDS) is an alternative drug delivery that offers various advantages. It can increase drugs' bioavailability, avoid hepatic first-pass metabolism, provide a non-invasive route for the patient, and reduce dosing frequency, thus improving patient compliance. In addition, TDDS can be self-administrated and generally inexpensive [8,9]. Moreover, because patients with hypertension need long term treatment, TDDS provide a more convenient and acceptable route [7]. Gels can provide an advantage in delivering valsartan via the transdermal route because gels tend to be softer, non-greasy, offer a cooling effect and provide a better drug release effect than other semisolid dosage forms [10]. Carbopol is one of the polymers that can be used as a gel base because it can increase the viscosity of preparations with low concentrations and can be combined with many active substances. A study by [11] showed that Carbopol-based gel exhibits better drug release than other gel bases.

VAL is a promising candidate to be successfully delivered by transdermal route by having a low molecular weight (434.5 Da) and melting point below 200°C (116-117°C) [12]. Moreover, there are no reports of skin irritation caused by VAL [13]. Nevertheless, the log partition coefficient (log P) of VAL is too high (4.5), which limits its permeation through the skin. High log P could cause the drug to be retained in the membrane longer, resulting the inhibition of drugs from being absorbed into the systemic circulation [14]. Besides, the existence of the human skin barrier (*stratum corneum*) is also one of the main challenges in developing transdermal formulation [15]. The barrier effect of the *stratum corneum* is most likely caused by its high density and low hydration [8]. Various strategies have been utilized to improve transdermal drug delivery systems, one of which is solid microneedles.

Solid microneedles (SMNs) are a transdermal permeation enhancement technique that comes from micrometre-sized needles. These needles are long enough to penetrate the stratum corneum but too short of stimulating pain receptors that are located in the dermis. SMNs have been used as a pre-treating step before applying the transdermal dosage form [16]. It is a potential and painless method to enhance the permeation of drugs by creating micropores into the skin. Thus, drugs could easily permeate across the barrier [9,15]. The resulting micropores would close immediately after the SMNs are moved to prevent infection by pathogenic microorganisms [17].

To date, no studies have reported on combining solid microneedle and transdermal gel for delivering valsartan. Hence the present work was carried out to develop Carbopol-based valsartan transdermal gel and solid microneedle to improve its permeation through the skin.

2. Material and Methods

2.1. Chemicals

Valsartan (Purity: >98.0%(T)(HPLC) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Carbopol 940 and DMDM Hydantoin were purchased from Sigma-Aldrich Pte Ltd. (Singapore, Singapore). Solid microneedles (Dermarollers®) were obtained from SQY® (Guangdong, China). Other materials were analytical grade

2.2. Preparation of transdermal gel

Various VAL transdermal gel was prepared using Carbopol as gel base [18]. Four different valsartan transdermal gel formulations were prepared using various concentrations of Carbopol, as shown in **Table 1**. The gel was made by dispersing Carbopol in distilled water and then left for 24 hours until it was completely hydrated. Triethanolamine (TEA) was added to the obtained dispersion, followed by homogenization at 1000 rpm for 15 minutes. After that, VAL was dissolved in 95% ethanol. The valsartan solution and DMDM hydantoin were then added to the gel and homogenized at 1000 rpm for 15 minutes until homogenous.

Table 1. Valsartan transdermal gel formulation composition

Compositions	%Composition (w/w)			
	F1	F2	F3	F4
Valsartan	1	1	1	1
Ethanol 95%	10	10	10	10
Carbopol 940	0.75	1	1.25	1.5
Triethanolamine	2.25	3	3.75	4.5
DMDM Hydantoin	0.1	0.1	0.1	0.1
Distilled water	ad 100	ad 100	ad 100	ad 100

2.3. pH measurement

pH measurement was conducted using a digital pH meter. The glass electrode was entirely soaked into the gel formulation at room temperature. This measurement was done in triplicate [19].

2.4. Viscosity study

The viscosity of gel was determined using Brookfield viscometer with suitable spindle speed and size [19]. In this study, the evaluation was performed at room temperature at 50 rpm using 50 g of sample, spindle number 7 and a total time of 60 s for one measurement.

2.5. Spreadability test

One of the ideal criteria required for the gel is it should possess good spreadability. The spreadability test of the gel was carried out by placing 0.5 g gel within a pre-recorded diameter of circle on a glass plate with a diameter of 25 cm. A second glass plate having the same dimension was placed over it. A weight of 500 g was allowed to rest on the upper glass plate for 5 minutes. The increase in diameter was measured and noted as spreadability (in cm) [20].

2.6. Extrudability test

Extrudability is an important parameter for evaluating gel formulation. Extrudability indicates the force that is required to remove the formulation from the tube. This test was done using the following method: The aluminium tube was filled with a known amount of formulation, and the tube was sealed by crimping both ends. The tube was placed between two glass slides. A weight of 500 g was applied to the top of the glass. The cap was removed, and the amount of the extruded gel was collected and noted. The

extrudability of the formulation was calculated using the following equation: [21]

$$E = W/A$$

Where, E is extrudability, W is applied weight to extrude the formulation, and A is the area.

2.7. Gel strength determination

The gel strength was determined using the following method: a 100 ml borosilicate graduated measuring cylinder (diameter 1.5 inches) was filled with 60 g of the gel. A disc (diameter = 3 cm; thickness = 3 mm) mounted with 30 g of weight was placed above the gel surface. The time taken (in seconds) by the disc to sink 5 cm into the gel from the surface was measured as gel strength [22].

2.8. Drug content recovery

The drug content recovery was conducted by weighing 1 g of gel (containing 10 mg of valsartan) and putting it in the 25 mL volumetric flask. The volume was then adjusted using ethanol 95%. The absorbance was measured triplicate using a validated UV-Vis Spectrophotometer at 231.4 nm. The determination of the concentration of VAL was performed by plotting the absorbance obtained to the calibration curve made from the standard solution of VAL in the concentration ranging from 0.5 µg/mL to 32 µg/mL.

2.9. In vitro drug release evaluation

The *in vitro* release profile of valsartan gel was measured using the membrane dialysis method [23,24]. Spectra-Por®, pore size of 12,000–14,000 MWCO dialysis membrane (Spectrum Medical Industries, CA, USA) was prepared and immersed in PBS solution for 24 hours. One gram of valsartan gel was accurately weighed and placed in the

dialysis membrane (25 x 50 mm) using spatula. The dialysis membrane was sealed and put into a 100 mL Duran bottle filled with PBS pH 7.4 and was placed on a magnetic stirrer. The temperature was maintained at 37±1°C. The rpm was set at 100 rpm during the evaluation. An aliquot of 1 ml was sampled at predetermined time intervals. The sampled solution was analyzed using a UV-Vis spectrophotometer at a wavelength of 231.4 nm. The result obtained was then calculated to determine the amount of drug released. The experiment was carried out in triplicates.

2.10. *In vitro* skin occlusivity evaluation

The occlusion properties of VAL gel were evaluated using the previously described method [25]. The test was carried out using Whatman filter paper (cut off 2.5 µm). 250 mg of the gel was applied to the Whatman filter paper and was placed on the top of a beaker filled with 50 mL of distilled water. The temperature was maintained at skin temperature (32°C). Using a similar method, a beaker filled with 50 mL distilled water was covered with Whatman filter paper without gel working as the negative control. At predetermined time intervals (6h, 24h, 48h), the systems were weighed to measure the water loss. The occlusivity (F_0) of the samples was determined using the following equation:

$$F_0 = \frac{W_0 - W_1}{W_0} \times 100\%$$

Where W_0 is the water loss of the control system and W_1 is the water loss of the gel. No occlusion is indicated by zero value of F_0 , and maximal occlusion is indicated by 100 values of F_0 .

2.11. *Ex vivo* permeation study

The *ex vivo* permeation study was conducted using vertical Franz cell and abdominal skin from Wistar rats. In order to obtain the skin, the rat's hair was removed using an electrical shaver and hair removal cream. Following this, the skins were collected after the rats were sacrificed. The full-thickness skins were collected by removing the fats attached to the skins. The skin was washed using PBS prior to the experiment. The diameters of the skins were 2.65 ± 0.18 mm. The skin collection was ethically permitted by the Health Ethical Committee at the Faculty of Medicine, Hasanuddin University, Indonesia. The skin was pre-treated using various lengths of solid microneedles (Dermaroller®), namely 0.5, 1.0, and 1.5 mm, creating 100 pores in the skin. The skin with a surface area of 4.9 cm^2 was placed between the donor and receptor compartment to facilitate the valsartan permeation. The receptor compartment was filled with PBS pH 7.4. The temperature during the study was maintained at $37 \pm 1^\circ\text{C}$. Afterwards, 1 g of gel formulation (equal to 10 mg of VAL) was applied to the skin. At each predetermined time, an aliquot of 1 mL was taken from the receptor compartment and then replaced by 1 mL of the fresh release medium to maintain the sink condition. All samples were analyzed by using UV-Vis spectrophotometry at a wavelength of 231.4 nm. The same method was used to compare abdominal skin without pre-treatment [26]. Additionally, the integrity of the skin following the application of Dermaroller® was evaluated using Fourier transform infrared (FTIR) spectroscopy [27]. At the end of *ex vivo* permeation study, the skin was washed and analyzed using FTIR. Untreated skin sample was also analyzed as a control.

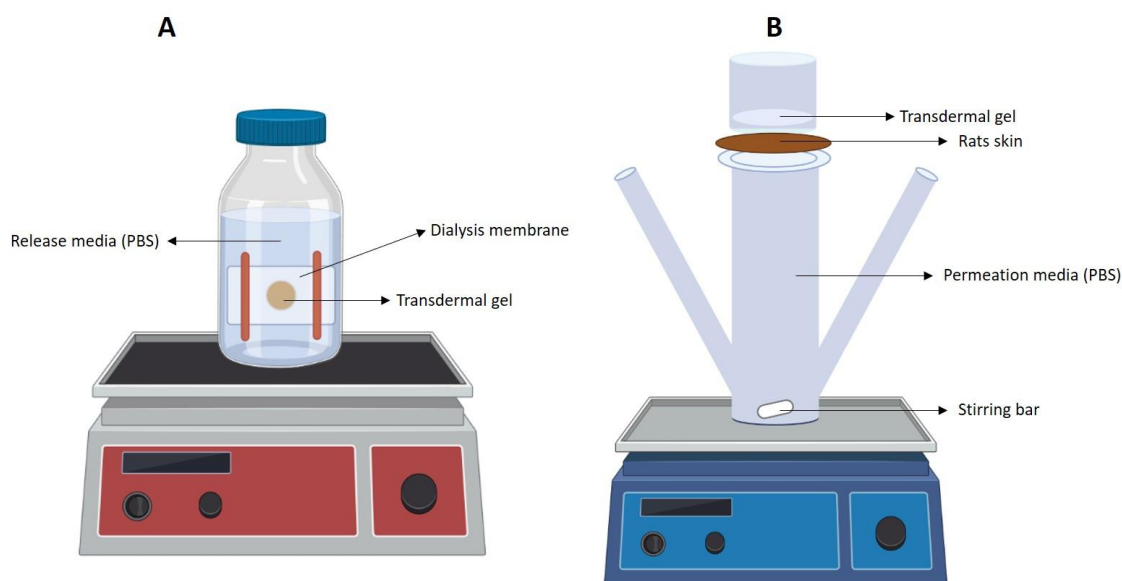


Fig. 1 Schematic procedure of *in vitro* release study (A) and *ex vivo* permeation study (B) of VAL from transdermal gel

2.12. *In vitro* hemolysis

The safety and biocompatibility of VAL gel were determined from *in vitro* hemolytic activity study [28,29]. This study was carried out using a fresh blood sample obtained from Wistar rats. Red blood cells (RBC) were separated from the plasma through centrifugation at 2000 rpm for 20 min. The RBC was washed three times using PBS, followed by vortex and centrifugation at 2000 rpm for 10 min. The washed RBC was resuspended in PBS to obtain a concentration of 10% v/v. An aliquot of 100 μ L of tested samples was added to 900 μ L RBC and incubated at 37°C for 1 h, followed by centrifugation at 7000 rpm for 10 min. Finally, the obtained supernatant was analyzed using UV-Vis Spectrophotometer at 540 nm. As a positive and negative control, blood samples were added with PBS and water, respectively. Calculation of the hemolysis percentage was done using the following equation:

$$\text{Hemolysis (\%)} = \frac{(\text{OD test sample}) - (\text{OD negative control}) \times 100}{(\text{OD positive control}) - (\text{OD negative control})}$$

3. Results and Discussion

3.1. Preparation of VAL Transdermal Gel

This study was conducted as a proof of concept to develop a combination approach of solid microneedles and transdermal gel for VAL delivery. In this study, VAL was formulated into the transdermal gel using Carbopol 940 as the polymer. Carbopol 940 is a hydrophilic polymer used in gel preparation. Carbopol would form acidic dispersion and undergo hydration when it was mixed with water. The addition of base is needed in order to obtain the viscous gel. To achieve this, triethanolamine (TEA) was added to neutralize the Carbopol dispersion [30]. A total of four VAL transdermal gel formulations were developed and evaluated to obtain the optimum formulation.

3.2. pH measurement

pH value is one of the important parameters that need to be considered. The pH of healthy skin is reported to be around 4.1 – 5.8 [31]. pH value plays an important role in maintaining skin barrier function. Acidic pH of the skin is responsible for controlling the presence of skin microflora [32,33]. The barrier integrity of the skin was also affected by the pH value [34]. In this study, we used Carbomer as the main gelling agent. It has been reported by numerous studies that the gel formation prepared by Carbomer could occur effectively at neutral pH (around 7) [35,36]. Therefore, the addition of TEA could neutralize the pH of the gels. The result showed that pH values of VAL transdermal gel were between 7.41 ± 0.01 and 7.56 ± 0.01 (Table 2). Although the obtained results were not in the range of the normal skin pH, a study conducted by Alberti et al., 2005 stated that transdermal formulations with pH values of 4-10 are still well tolerated. In line with this study, a previous study showed that a pH value of 7 did

not result in skin irritation [38]. Accordingly, the developed VAL transdermal gels were considered safe and could not possibly cause irritation to the skin.

3.3. Viscosity and spreadability study

The viscosity of VAL transdermal gel was measured and noted. The obtained results are depicted in **Fig. 2 (A)**. The higher Carbopol concentration used in the formulation would result in higher viscosity. Dispersion and hydration of Carbopol in water result in acidic dispersion with low viscosity which a small portion of carboxyl groups present on the polymer chain dissociate. Neutralization using base would increase viscosity due to further dissociation of carboxyl group on the polymer and internal repulsion between polymer strands which causes uncoiling and expansion, resulting in swelling and gel formation [30,39]. It was found that higher Carbopol concentration led to statistically different viscosity of the gel ($p < 0.05$). Additionally, it was also important to note that the pH of the formulations also increased with the increase of Carbomer and TEA concentration. As previously explained, the neutralization could affect the viscosity of the gels. The pH of F4 was found to be highest, followed by F3, F2 and F1. This could result in the higher viscosity of the formulation following the pH values of each formulation. VAL transdermal gels were then evaluated for their spreadability, as good spreadability is one of the ideal criteria for good gels. The spreadability of VAL transdermal gel showed in **Fig. 2 (B)**. The spreadability could improve patient compliance and helps in the uniform application of gels. Spreadability value also affects the therapeutic efficacy of gel formulation [13,20]. Higher spreadability value showed by VAL transdermal gel containing Carbopol with low concentration. This implies that viscosity and spreadability value were inversely related.

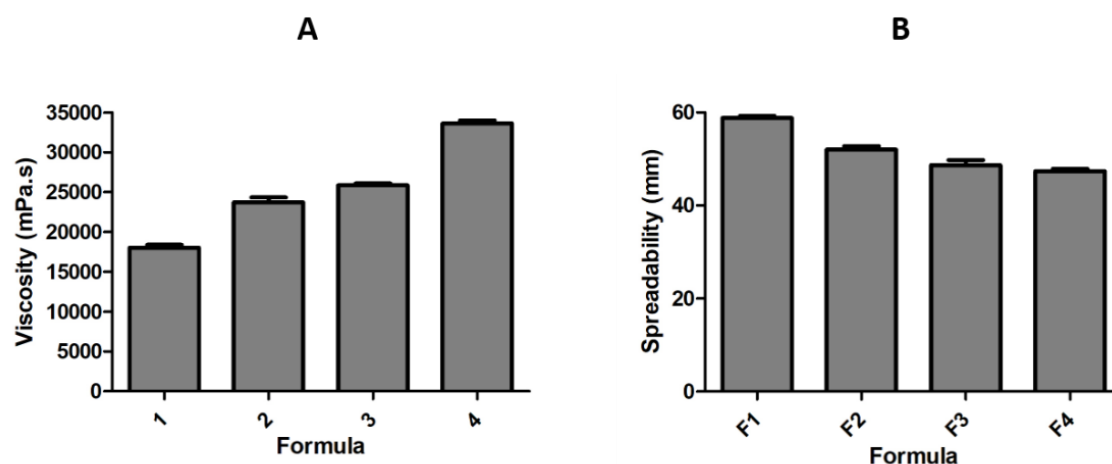


Fig. 2 The result of (A) viscosity test and (B) spreadability test of VAL transdermal gel. (mean $\pm$ SD, n=3)

3.4. Extrudability test

Extrudability is an important parameter for evaluating gel formulation. This test indicates the force required to remove the formulation from the tube. The more gels extruded, the better extrudability since only lower energy was needed to extrude the gel from the aluminium tube. Extrudability values of VAL transdermal gel can be seen in Table 2. F1, which contains 0.75% Carbopol, exhibited the highest extrudability, while F4, containing 1.5% Carbopol, showed the lowest extrudability. Despite the difference, the extrudability values among VAL transdermal gels were statistically insignificant ($p > 0.05$). The result showed that VAL transdermal gel exhibited good extrudability [21,40].

3.5. Gel strength determination

The result of gel strength determination is shown in Table 2. It plays a crucial role as low gel strength could lead to loss of gel integrity and fast gel removal, while high gel strength indicates very stiff gel. Discomfort when used by patients and difficulties in

spreading the gel, **are** also caused by high gel strength. Optimal gel strength is **characterised** by a value of 25-50 seconds. All developed formulations **showed** gel strength values that fall within the optimal range indicating VAL transdermal gel exhibits **good** gel strength. Furthermore, gel strength values were found to be not related to **the** Carbopol concentration used in the formulas as it did not statistically different ($p > 0.05$) [41,42].

3.6. Drug content recovery

Drug content recovery test is carried out to determine the amount of VAL in the final product. The result in **Table 2.** showed drug content in the VAL transdermal gels **was** between 101.19-103.03 %, which implies the method used in gel fabrication produced **a** homogeneous formulation. Moreover, it confirms that **the fabrication method did not affect the amount of VAL in the formulation.** The results obtained also were fall within the requirement **of** drug content recovery set by **the** International Council for Harmonization (ICH), which was in **the** range of 95-105%. [43].

Table 2. Result of pH, extrudability, gel strength, and drug content recovery test of VAL transdermal gel formulations (mean $\pm$ SD, n=3)

Formula	pH	Extrudability (g/cm ²)	Gel strength (s)	Drug content recovery (%)
F1	7.41 $\pm$ 0.01	1.49 $\pm$ 0.18	36.20 $\pm$ 3.93	101.19 $\pm$ 0.13
F2	7.42 $\pm$ 0.01	1.45 $\pm$ 0.20	38.30 $\pm$ 4.09	102.67 $\pm$ 0.44
F3	7.50 $\pm$ 0	1.37 $\pm$ 0.17	39.88 $\pm$ 3.98	103.04 $\pm$ 0.56
F4	7.56 $\pm$ 0.01	1.34 $\pm$ 0.16	40.31 $\pm$ 4.58	103.41 $\pm$ 0.13

3.7. In vitro drug release evaluation

The *in vitro* drug releases of VAL transdermal gels **are** depicted in **Fig. 3 (A).**

Specifically, the data showed that 10.78 ± 0.46 mg, 9.64 ± 0.35 mg, 9.08 ± 0.43 mg, and 8.44 ± 0.25 mg of VAL were released from F1, F2, F3, and F4, respectively, after 8 h. Specifically, it was found that in the first 2 hours, all formulations exhibited burst release, showing the release percentage of around 80%. Afterwards, the sustained release manner was shown in the following hours. The burst release occurred possibly due to the presence of VAL on the outer surface of the gel or in the gel surface layers voids. This phenomenon was also reported previously [44]. Analyzed statistically, the VAL released from gel formulation were significantly different ($p < 0.05$), indicating that Carbopol concentration affected the release of VAL. A higher amount of VAL released was observed as the concentration of Carbopol decreased in the formulation. This finding was in line with a previous study [45]. This was because increasing Carbopol concentration would form high viscosity of the polymeric system, as the carboxyl groups dissociate, leading to electrostatic repulsion, resulting in the tight and hard gel. The formed gel would have a more rigid structure. This would hinder the diffusion of drug from the polymer matrix and decrease the amount of VAL released [46,47].

3.8. *In vitro* skin occlusivity evaluation

This test was performed to evaluate the ability of VAL transdermal gel formulation to maintain the hydration characteristic of the skin. It indicates the prevention of water loss by the formulations. The results are presented in **Fig. 3 (B)**. All formulations showed high occlusivity. The occlusivity of formulations would affect the amount of drugs in the skin. Higher occlusivity could increase skin hydration which facilitates drug penetration. This is due to the reduction of corneocyte gaps [48,49]. Additionally, it was also observed that there was no significant difference ($p > 0.05$) in the occlusivity values between all formulations with the control formulations, as shown **Fig. 3 (C)**.

Accordingly, the addition of VAL did not alter the occlusivity of Carbomer-based gels. Several studies have shown the benefits of the occlusivity of topical preparation in the transdermal delivery system. The occlusivity of the preparations did not cause damage and irritation to the skin [50–52]. Importantly, the values obtained in our study were found to be comparable with several previous reports. Accordingly, it might be hypothesized that the occlusivity properties of the gel did not possibly cause any damage in the skin.

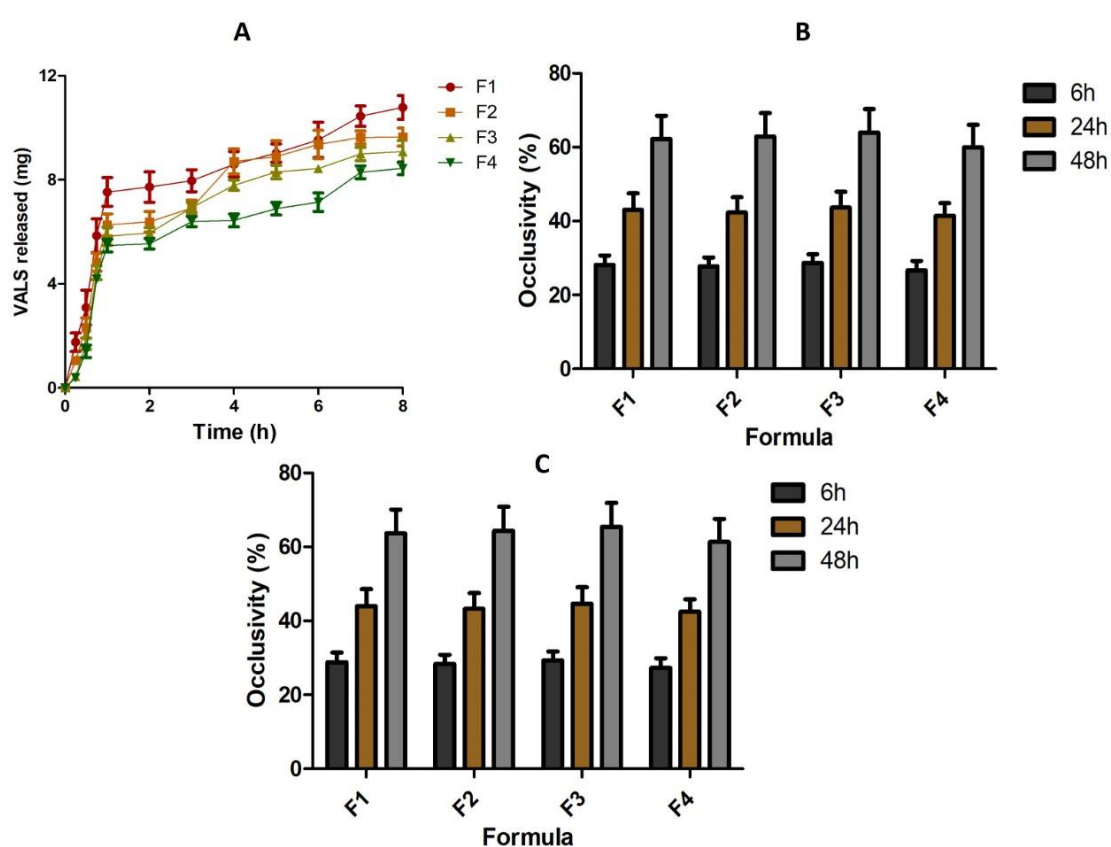


Fig.3 (A) *In vitro* release profile, (B) *in vitro* occlusivity of VALS transdermal gel and *in vitro* occlusivity of blank transdermal gel (C) (mean $\pm$ SD, n=3).

3.9. *Ex vivo* permeation study

To evaluate the permeation of VAL from transdermal gel, an *ex vivo* permeation study was carried out. This study was performed using abdominal skin from Wistar rats. The skin was pre-treated using Dermalroller® (solid microneedle) with various needle

lengths, namely 0.5 mm, 1 mm, and 1.5 mm. The illustration of the administration of Dermarollers® and the pores created in the skin are shown in **Fig. 4**. The permeation profile of VAL transdermal gel is illustrated in **Fig. 5**. All formulations exhibited higher permeation when it was pre-treated using Dermaroller® compared to permeation from non-treated abdominal skin ($p < 0.05$). The mechanism behind it was “poke and patch”. Micropores were formed when Dermaroller® was applied to the skin surface as the microneedle penetrated the skin barrier (*stratum corneum*). Thus, when the transdermal gel was applied, the drug could easily pass the barrier and penetrate into the skin [15,53]. Furthermore, the various microneedle lengths also affected the drug permeation profile in all formulations. It was clearly observed that the longer length of microneedle exhibits a higher permeation profile of VALS due to deeper penetration of the drug [54].

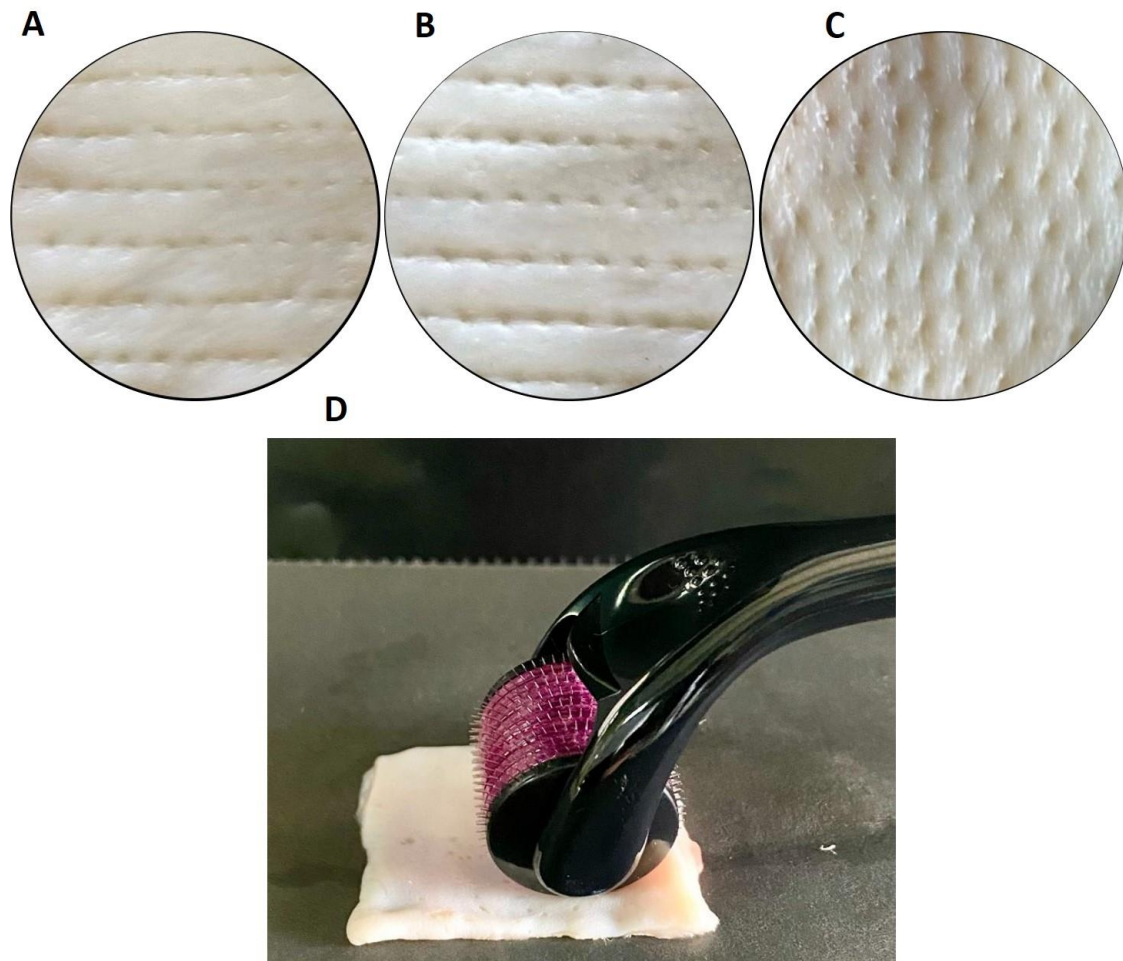


Fig. 4. The representative images of the administration of Dermarollers® (A) and the pores created in the skin followong the administration of Dermarollers® with lenghts of 0.5 mm (B), 1 mm (C) and 1.5 mm (D)

Without the use of MNs, the percentages of permeation of VALS were found to be $5.44 \pm 0.01\%$, $4.98 \pm 0.02\%$, $4.61 \pm 0.01\%$ and $4.49 \pm 0.02\%$ of VALS permeated from F1, F2, F3 and F4, respectively. As shown in Fig.3, pre-treated with Dermaroller® 0.5 mm was able to enhance the percentages of permeation to $14.33 \pm 0.04\%$ for F1, $13.92 \pm 0.07\%$ for F2, $12.05 \pm 0.01\%$ for F3, and $10.63 \pm 0.09\%$ for F4. Furthermore, the permeation percentages were significantly improved ($p < 0.05$) after the use of Dermarollers® with lengths of 1 mm, and 1.5 mm, respectively. The results showed that $15.37 \pm 0.08\%$, $14.96 \pm 0.08\%$, $12.44 \pm 0.04\%$ and $11.06 \pm 0.17\%$ of VAL permeated

from transdermal gels F1, F2, F3 and F4, respectively after the administration of 1 mm Dermaroller®. Finally, the use of 1.5 mm Dermarolles® increased the permeation percentages up to $16.92 \pm 0.04\%$ for F1 $15.68 \pm 0.09\%$ for F2, $14.77 \pm 0.02\%$ for F2 and $12.97 \pm 0.15\%$ for F3. Analyzed statistically, there was a significant different ($p < 0.05$) on drug permeation against various length of microneedle. In line with the *in vitro* release study, the increase of Carbopol concentration could decrease the permeation of VAL from the formulation due to the high viscosity of the polymeric system as a result of the carboxyl groups dissociating and electrostatic repulsion, producing a tight gel structure [46,47]. Therefore, F1 was considered as the optimum formula. Furthermore, the results showed that the permeation coefficient values of VALS permeated from F1 were observed to be 0.032 ± 0.002 cm/h for untreated skin, 0.047 ± 0.002 cm/h for the application of 0.5 mm Dermaroller®, 0.059 ± 0.003 cm/h for the application of 1 mm Dermaroller® and 0.065 ± 0.03 cm/h for the application of 1.5 mm Dermaroller®. As the permeation coefficient value increased with the increase of length needle, F1 combined with Dermaroller® 1.5 mm was chosen as the optimum treatment condition for VAL permeation. Several studies have previously reported that microneedles with a length of around 1.5 mm did not cause any discomfort when it was used by the patient [55–57].

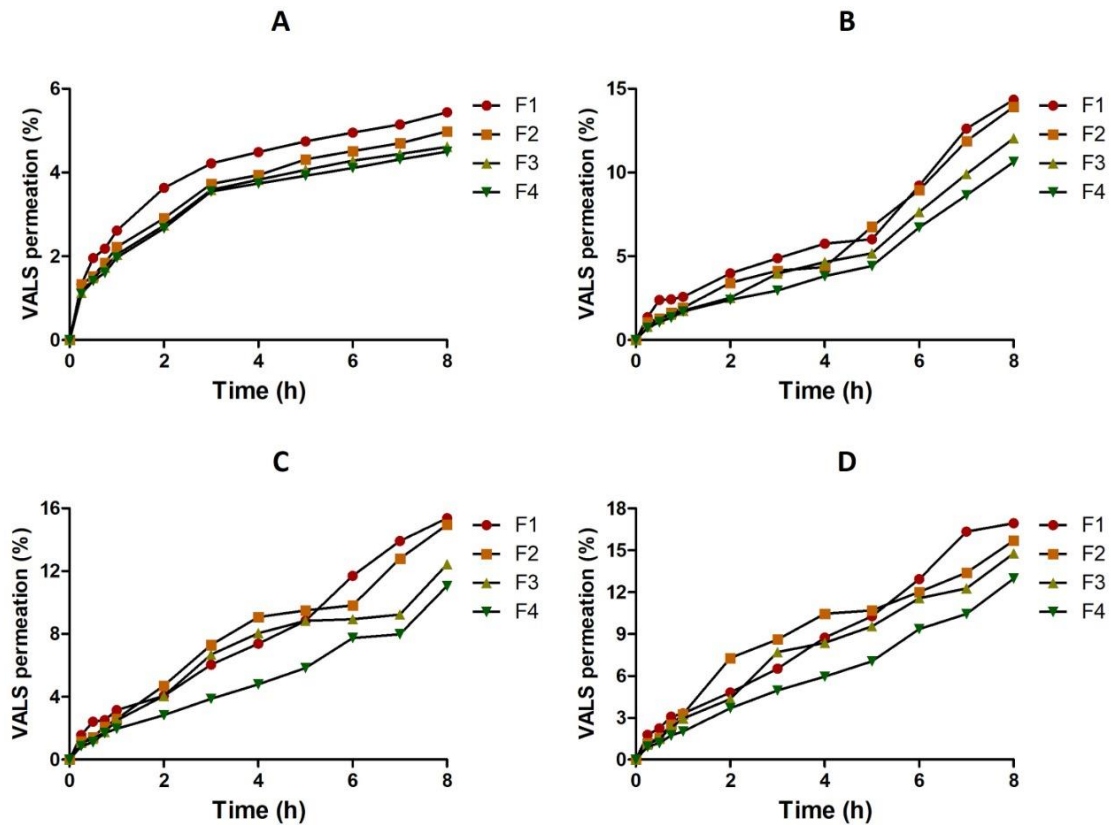


Fig. 5. *Ex vivo* permeation profile of VALS transdermal gel (A) without MNs, (B) with MNs 0.5 mm, (C) with MNs 1 mm, and (D) with MNs 1.5 mm (mean $\pm$ SD, $n=3$).

Following the integrity evaluation using FTIR (Fig. 5), it was found that the untreated skin showed noticeable peaks at 2902 cm^{-1} and 2756 cm^{-1} due to asymmetric hydrocarbon stretching and symmetric CH_2 stretching, respectively. The presence of a hydrocarbon area indicates the ceramide and the fatty acid contained in the *stratum corneum*. Moreover, other peaks were also observed at 1678 cm^{-1} and 1518 cm^{-1} due to the presence of the amide I and amide II bonds, respectively, in the corneocytes' keratin [27]. Importantly, all these peaks were also observed in all treated skins with negligible changes in intensity. Accordingly, the administration of Dermarollers® did not change the integrity of the skin.

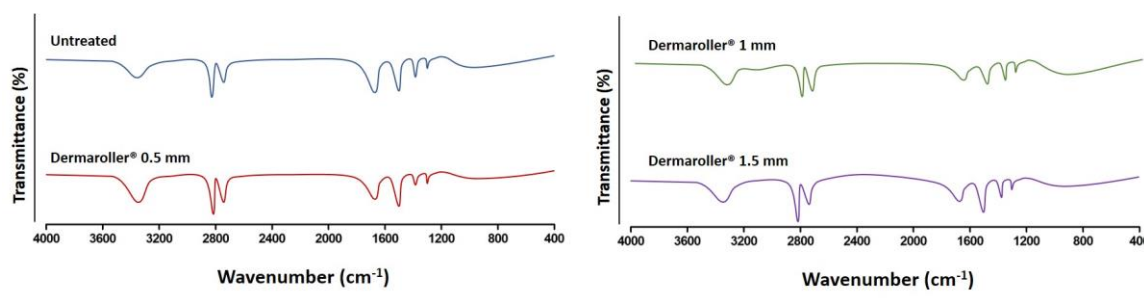


Fig. 5. FTIR spectrum of skin indicating the integrity following the administration of Dermaroller®

3.10 *In vitro* hemolysis

This test was carried out to determine the safety, biocompatibility, and initial toxicity of the developed formulation against erythrocytes. In this study, we evaluated the hemolysis ability of VAL from F1 as the optimum formulation. Hemolysis percentage values were showed in **Table 3**. All formulations had hemolysis percentage values between 0.98-3.19%. Hemolysis percentage values were previously reported to be safe when less than 5%. The result clearly indicates that VALS transdermal gel would be safe to use at the tested concentration [58,59].

Table 3. Hemolysis percentage of VALS transdermal gel (mean $\pm$ SD, n=3)

Concentration ($\mu\text{g/mL}$)	Hemolysis (%)			
	F1	F2	F3	F4
500	2.97 $\pm$ 0.28	3.05 $\pm$ 0.30	3.08 $\pm$ 0.32	3.16 $\pm$ 0.34
50	2.23 $\pm$ 0.30	2.20 $\pm$ 0.21	2.03 $\pm$ 0.20	2.22 $\pm$ 0.28
5	0.98 $\pm$ 0.11	1.14 $\pm$ 0.12	1.05 $\pm$ 0.11	1.17 $\pm$ 0.14

The overall outcomes resulting from this investigation have proven the potential ability of solid microneedles to assist the transdermal delivery of VALS from polymeric

hydrogel to avoid the drawbacks of oral delivery. Our study suggested that the permeation of VALS from conventional transdermal hydrogel was significantly lower when compared to its combination with solid microneedles with no toxicity possibility. To show the efficacy of this combination, further *in vivo* studies should be considered to assess the pharmacokinetic, pharmacodynamic and skin irritation studies.

4. Conclusion

The present study focused on the development of Carbopol-based VALS transdermal gel and combination with microneedles. The developed formulations were found to produce gels with good physical characteristics. Furthermore, a combination with a microneedle (Dermaroller®) showed improved permeation of VALS into the skin significantly. Microneedles with a length of 1.5 mm exhibited the highest permeation compared to other lengths. Importantly, *in vitro* hemolysis study showed hemolysis percentage below 5%, indicating that VAL transdermal gels were considerably safe to use. This study successfully developed a new approach for VALS drug delivery to treat hypertension in the future. However, further studies are required to investigate the effectiveness of this approach, including *in vivo* pharmacokinetics and toxicity using suitable animal models.

References:

- [1] Oparil S, Acelajado MC, Bakris GL, et al. Hypertension. Nat Rev Dis Prim. 2019;4:139–148.
- [2] Bazzano LA, Pogribna U, Whelton PK. Hypertension. Int Encycl Public Heal. 2016;4:123–133.
- [3] Singh S, Shankar R, Singh GP. Prevalence and Associated Risk Factors of Hypertension: A Cross-Sectional Study in Urban Varanasi. Int J Hypertens.

2017;2017.

- [4] Forouzanfar MH, Afshin A, Alexander LT, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388:1659–1724.
- [5] Nixon RM, Müller E, Lowy A, et al. Valsartan vs. other angiotensin II receptor blockers in the treatment of hypertension: A meta-analytical approach. *Int J Clin Pract*. 2009;63:766–775.
- [6] 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;23:119–125.
- [7] Ahad A, Al-Mohizea AM, Al-Jenoobi FI, et al. Transdermal delivery of angiotensin II receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACEIs) and others for management of hypertension. *Drug Deliv*. 2016;23:579–590.
- [8] Alkilani AZ, McCrudden MTC, Donnelly RF. Transdermal drug delivery: Innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. *Pharmaceutics*. 2015;7:438–470.
- [9] Prausnitz MR, Langer R. Transdermal drug delivery. *Nat Biotechnol*. 2009;26:1261–1268.
- [10] Suryakumari C, Narender M, Umasankar K, et al. Formulation and Evaluation of Tacrolimus Transdermal Gel. *J Drug Deliv Ther*. 2019;9:110–118.
- [11] Patel J, Patel B, Banwait H, et al. Formulation and evaluation of topical diclofenac sodium gel using different gelling agent. *Int Journal Drug Dev Res*. 2011;3:156–164.
- [12] Iqbal B, Ali J, Baboota S. Recent advances and development in epidermal and dermal drug deposition enhancement technology. *Int J Dermatol*. 2018;57:646–660.
- [13] Ahad A, Aqil M, Ali A. Investigation of antihypertensive activity of carbopol valsartan transdermal gel containing 1,8-cineole. *Int J Biol Macromol*. 2014;64:144–149.

- [14] Liu X, Testa B, Fahr A. Lipophilicity and its relationship with passive drug permeation. *Pharm Res.* 2011;28:962–977.
- [15] Jung JH, Jin SG. Microneedle for transdermal drug delivery: current trends and fabrication. *J Pharm Investig.* 2021;
- [16] Waghule T, Singhvi G, Dubey SK, et al. Microneedles: A smart approach and increasing potential for transdermal drug delivery system. *Biomed Pharmacother.* 2019;109:1249–1258.
- [17] Ita K. Transdermal delivery of drugs with microneedles: Strategies and outcomes. *J Drug Deliv Sci Technol.* 2015;29:16–23.
- [18] Enggi CK, Isa HT, Sulistiawati S, 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].* 2021;609:121182. Available from: <https://doi.org/10.1016/j.ijpharm.2021.121182>.
- [19] Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. *Brazilian J Pharm Sci.* 2016;52.
- [20] Ahad A, Aqil M, Kohli K, et al. Design, formulation and optimization of valsartan transdermal gel containing iso-eucalyptol as novel permeation enhancer: Preclinical assessment of pharmacokinetics in Wistar albino rats. *Expert Opin Drug Deliv.* 2014;11:1149–1162.
- [21] Dudhipala N, Gorre T. Neuroprotective effect of ropinirole lipid nanoparticles enriched hydrogel for parkinson's disease: In vitro, ex vivo, pharmacokinetic and pharmacodynamic evaluation. *Pharmaceutics.* 2020;12:1–24.
- [22] Algahtani MS, Ahmad MZ, Ahmad J. Nanoemulsion loaded polymeric hydrogel for topical delivery of curcumin in psoriasis. *J Drug Deliv Sci Technol.* 2020;59:101847.
- [23] Permana AD, McCrudden MTC, Donnelly RF. Enhanced intradermal delivery of nanosuspensions of antifilaria drugs using dissolving microneedles: A proof of concept study. *Pharmaceutics.* 2019;11:346.
- [24] Permana AD, Paredes AJ, Volpe-Zanutto F, et al. Dissolving microneedle-

- mediated dermal delivery of itraconazole nanocrystals for improved treatment of cutaneous candidiasis. *Eur J Pharm Biopharm* [Internet]. 2020;154:50–61. Available from: <https://doi.org/10.1016/j.ejpb.2020.06.025>.
- [25] Malik DS, Kaur G. Nanostructured gel for topical delivery of azelaic acid: Designing, characterization, and in-vitro evaluation. *J Drug Deliv Sci Technol*. 2018;47:123–136.
- [26] Ahmed KS, Shan X, Mao J, et al. Derma roller® microneedles-mediated transdermal delivery of doxorubicin and celecoxib co-loaded liposomes for enhancing the anticancer effect. *Mater Sci Eng C* [Internet]. 2019;99:1448–1458. Available from: <https://doi.org/10.1016/j.msec.2019.02.095>.
- [27] Talib S, Ahmed N, Khan D, et al. Chitosan-chondroitin based artemether loaded nanoparticles for transdermal drug delivery system. *J Drug Deliv Sci Technol* [Internet]. 2021;61:102281. Available from: <https://doi.org/10.1016/j.jddst.2020.102281>.
- [28] Ananda PWR, Elim D, Zaman HS, et al. Combination of transdermal patches and solid microneedles for improved transdermal delivery of primaquine. *Int J Pharm* [Internet]. 2021;609:121204. Available from: <https://doi.org/10.1016/j.ijpharm.2021.121204>.
- [29] Qonita HA, Syafika N, Valensie V, et al. Development water in oil nanoemulsion of diethylcarbamazine for enhanced the characteristics for lymphatic targeting: A proof of concept study. *J Indian Chem Soc* [Internet]. 2022;99:100395. Available from: <https://doi.org/10.1016/j.jics.2022.100395>.
- [30] Barry BW, Meyer MC. The rheological properties of carbopol gels I. Continuous shear and creep properties of carbopol gels. *Int J Pharm*. 1979;2:1–25.
- [31] Proksch E. pH in nature, humans and skin. *J Dermatol*. 2018;45:1044–1052.
- [32] Paudel KS, Milewski M, Swadley CL, et al. Challenges and opportunities in dermal/transdermal delivery. *Ther Deliv*. 2010;1:109–131.
- [33] Lambers H, Piessens S, Bloem A, et al. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *Int J Cosmet Sci*. 2006;28:359–370.
- [34] Gorzelanny C, Mess C, Schneider SW, et al. Skin barriers in dermal drug

delivery: Which barriers have to be overcome and how can we measure them? *Pharmaceutics*. 2020;12:1–31.

- [35] Tripathi P, Kumar A, Jain PK, et al. Carbomer gel bearing methotrexate loaded lipid nanocontainers shows improved topical delivery intended for effective management of psoriasis. *Int J Biol Macromol* [Internet]. 2018;120:1322–1334. Available from: <http://dx.doi.org/10.1016/j.ijbiomac.2018.08.136>.
- [36] Zhang Y, Ng W, Hu J, et al. Formulation and in vitro stability evaluation of ethosomal carbomer hydrogel for transdermal vaccine delivery. *Colloids Surfaces B Biointerfaces* [Internet]. 2018;163:184–191. Available from: <http://dx.doi.org/10.1016/j.colsurfb.2017.12.031>.
- [37] Alberti I, Grenier A, Kraus H, et al. Pharmaceutical development and clinical effectiveness of a novel gel technology for transdermal drug delivery. *Expert Opin Drug Deliv*. 2005;2:935–950.
- [38] Al-Suwayeh SA, Taha EI, Al-Qahtani FM, et al. Evaluation of skin permeation and analgesic activity effects of carbopol lornoxicam topical gels containing penetration enhancer. *Sci World J*. 2014;2014.
- [39] Mastropietro D, Park K, Omidian H. Polymers in oral drug delivery. *Compr Biomater II*. 2017. p. 430–444.
- [40] Gupta R, Gupta G Das. Formulation development and evaluation of anti-inflammatory potential of cordia obliqua topical gel on animal model. *Pharmacogn J*. 2017;9:s93–s98.
- [41] Mahajan HS, Gattani S. In situ gels of Metoclopramide Hydrochloride for intranasal delivery: In vitro evaluation and in vivo pharmacokinetic study in rabbits. *Drug Deliv*. 2010;17:19–27.
- [42] Morsi N, Ibrahim M, Refai H, et al. Nanoemulsion-based electrolyte triggered in situ gel for ocular delivery of acetazolamide. *Eur J Pharm Sci*. 2017;104:302–314.
- [43] Walfish S. A statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. *BioPharm Int*. 2006;19:28–36.
- [44] Khan S, Minhas MU, Tekko IA, et al. Evaluation of microneedles-assisted in situ depot forming poloxamer gels for sustained transdermal drug delivery. *Drug*

Deliv Transl Res. 2019;9:764–782.

- [45] Li S, Lin S, Daggy BP, et al. Effect of HPMC and Carbopol on the release and floating properties of Gastric Floating Drug Delivery System using factorial design. *Int J Pharm.* 2003;253:13–22.
- [46] Suhail M, Wu PC, Minhas MU. Using carbomer-based hydrogels for control the release rate of diclofenac sodium: Preparation and in vitro evaluation. *Pharmaceuticals.* 2020;13:1–17.
- [47] Nakanishi T, Kaiho F, Hayashi M. Improvement of drug release rate from Carbopol 934P formulation. *Chem Pharm Bull.* 1998;46:171–173.
- [48] Ammar HO, Ghorab MM, Mostafa DM, et al. Folic acid loaded lipid nanocarriers with promoted skin antiaging and antioxidant efficacy. *J Drug Deliv Sci Technol.* 2016;31:72–82.
- [49] Permana AD, Utami RN, Courtenay AJ, et al. 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.* 2020;205:111846.
- [50] Shrotriya S, Ranpise N, Satpute P, et al. Skin targeting of curcumin solid lipid nanoparticles-engrossed topical gel for the treatment of pigmentation and irritant contact dermatitis. *Artif Cells, Nanomedicine Biotechnol* [Internet]. 2018;46:1471–1482. Available from: <https://doi.org/10.1080/21691401.2017.1373659>.
- [51] Shrotriya SN, Ranpise NS, Vidhate B V. Skin targeting of resveratrol utilizing solid lipid nanoparticle-engrossed gel for chemically induced irritant contact dermatitis. *Drug Deliv Transl Res* [Internet]. 2017;7:37–52. Available from: <http://dx.doi.org/10.1007/s13346-016-0350-7>.
- [52] Wavikar P, Vavia P. Nanolipidgel for enhanced skin deposition and improved antifungal activity. *AAPS PharmSciTech.* 2013;14:222–233.
- [53] Avcil M, Çelik A. Microneedles in drug delivery: Progress and challenges. *Micromachines.* 2021;12:1–15.
- [54] Tobin K V., Fiegel J, Brogden NK. Thermosensitive gels used to improve

microneedle-assisted transdermal delivery of naltrexone. *Polymers (Basel)*. 2021;13:1–15.

- [55] Kim YC, Park JH, Prausnitz MR. Microneedles for drug and vaccine delivery. *Adv Drug Deliv Rev* [Internet]. 2012;64:1547–1568. Available from: <http://dx.doi.org/10.1016/j.addr.2012.04.005>.
- [56] Chu S, Foulad DP, Mesinkovska NA. Safety Profile for Microneedling: A Systematic Review. *Dermatologic Surg*. 2021;47:1249–1254.
- [57] Salloum A, Bazzi N, Maalouf D, et al. Microneedling in vitiligo: A systematic review. *Dermatol Ther*. 2020;33:1–7.
- [58] Greco I, Molchanova N, Holmedal E, et al. Correlation between hemolytic activity , cytotoxicity and systemic in vivo toxicity of synthetic antimicrobial peptides. *Sci Rep*. 2020;1–13.
- [59] Zhou HY, Zhang YP, Zhang WF, et al. Biocompatibility and characteristics of injectable chitosan-based thermosensitive hydrogel for drug delivery. *Carbohydr Polym*. 2011;83:1643–1651.

Ref.: Ms. No. JBS-D-22-00297

Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation

Journal of Biomaterials Science: Polymer Edition

Response to Reviewers

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

Reviewer #2:

The manuscript is very well written with organized experimentation section. It touches all aspects of formulation and analytical testing. However, I would recommend major additions which can further add to quality of manuscript.

Response to Reviewer

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

Section 2.2 – What was the function of DMDM hydantoin in formulation and how did it add to the value of the transdermal gel?

Response:

We thank the reviewer for the question. DMDM hydantoin was used as a preservative. As it is in the liquid form, it was added into the gel base with the valsartan solution, as explained in the revised manuscript.

Section 2.4 – Please discuss what speed and spindle were used to measure viscosity. Additionally, viscosity is also impacted by the temperature, what was the temperature of measurement?

Response:

We thank the reviewer for the suggestion. As a result, we have added the explanation in the revised manuscript. The evaluation was performed at room temperature at 50 rpm using 50 g of sample, spindle number 7 and a total time of 60 s for one measurement.

Section 2.5 – what was the diameter of glass plate?

Response:

We thank the reviewer for the question. The diameter of glass plate was 25 cm. We have added the explanation in the revised manuscript.

Section 2.8 – How was drug content calculated – against a standard or calibration curve? Please add this detail.

Response:

We thank the reviewer for the question. The determination of the concentration of VAL was performed by plotting the absorbance obtained to the calibration curve made from the standard

solution of VAL in the concentration ranging from 0.5 µg/mL to 32 µg/mL. We have added the explanation in the revised manuscript.

Section 2.9 – Please add the pore size of membrane. Please add dimensions of the membrane.

Response:

We thank the reviewer for the suggestion. The *in vitro* release profile of valsartan gel was measured using the membrane dialysis method (Permana et al., 2020a, 2019). Spectra-Por®, pore size of 12,000–14,000 MWCO dialysis membrane (Spectrum Medical Industries, CA, USA) was prepared and immersed in PBS solution for 24 hours. One gram of valsartan gel was accurately weighed and placed in the dialysis membrane (25 x 50 mm) using spatula. We have included these in the revised manuscript.

“one gram of valsartan gel was then dispersed on the cellophane membrane.” Please specify dispersed – was it spread?

Response:

We thank the reviewer for the suggestion. One gram of valsartan gel was accurately weighed and placed in the dialysis membrane. We have included this in the revised manuscript.

Please provide a figure of the experimental set up – will help the text.

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

How many number of replicates were performed?

Response:

We thank the reviewer for the suggestion. The experiment was carried out in triplicates. We have now included this in the revised manuscript.

Section 2.11 – Please add a figure of Franz diffusion cell setup

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

Can you please specify the details of skin – thickness, hairless, full thickness, how many pores were made from Dermaroller on the skin?

Response:

We thank the reviewer for the suggestion. The *ex vivo* permeation study was conducted using vertical Franz cell and abdominal skin from Wistar rats. In order to obtain the skin, the rat's hair was removed using an electrical shaver and hair removal cream. Following this, the skins were collected after the rats were sacrificed. The full-thickness skins were collected by removing the fats attached to the skins. The skin was washed using PBS prior to the experiment. The diameters of the skins were 2.65 ± 0.18 mm. The skin collection was ethically permitted by the Health Ethical Committee at the Faculty of Medicine, Hasanuddin University, Indonesia. We have now included these in the revised manuscript.

Was any integrity testing performed on the skin after use of Dermaroller?

Response:

We thank the reviewer for the suggestion. We have performed the skin integrity evaluation and have included the results in the revised manuscript.

What was the surface area of diffusion?

Response:

We thank the reviewer for the question. The diffusion area was 4.9 cm^2 . We have now included this in the revised manuscript.

What was the quantity of gel on the donor membrane?

Response:

We thank the reviewer for the suggestion. In this study, 1 g of gel formulation (equal to 10 mg of VAL) was applied to the skin. We have now included this in the revised manuscript.

Section 3.2 – please add description on why pH was adjusted to 7.4 when the same paragraph mentions pH of skin is 4.1 – 5.8.

Response:

We thank the reviewer for the suggestion. We have rephrased the explanations, as follows:

The pH of healthy skin is reported to be around 4.1 – 5.8 (Proksch, 2018). pH value plays an important role in maintaining skin barrier function. Acidic pH of the skin is responsible for controlling the presence of skin microflora (Lambers et al., 2006; Paudel et al., 2010). The barrier integrity of the skin was also affected by the pH value (Gorzalanny et al., 2020). In this study, we used Carbomer as the main gelling agent. It has been reported by numerous studies that the gel formation prepared by Carbomer could occur effectively at neutral pH (around 7) (Tripathi et al., 2018; Zhang et al., 2018). Therefore, the addition of TEA could neutralize the pH of the gels. The result showed that pH values of VAL transdermal gel were between 7.41 ± 0.01 and 7.56 ± 0.01 (**Table 2**). Although the obtained results were not in the range of the normal skin pH, a study conducted by Alberti et al., 2005 stated that transdermal formulations with pH values of 4-10 are still well tolerated. In line with this study, a previous study showed that a pH value of 7 did not result in skin irritation (Al-Suwayeh et al., 2014). Accordingly, the developed VAL transdermal gels were considered safe and could not possibly cause irritation to the skin.

We have now included this in the revised manuscript.

Section 3.3 – Paragraph mentions that neutralization increases the viscosity. The pH of F1 was recorded at 7.41 while pH of F4 is at 7.56. Table 1 shows pH of formulation from F1-F2-F3-F4

increases – could this be the cause of high viscosity rather than increase in carbomer content – discussion on this would be helpful.

Response:

We thank the reviewer for the suggestion. As a result, we have included the explanations, as follows:

Additionally, it was also important to note that the pH of the formulations also increased with the increase of Carbomer and TEA concentration. As previously explained, the neutralization could affect the viscosity of the gels. The pH of F4 was found to be highest, followed by F3, F2 and F1. This could result in the higher viscosity of the formulation following the pH values of each formulation.

We have now included this in the revised manuscript.

Section 3.7 – according to the experimental setup “one gram of valsartan gel was then dispersed on the cellophane membrane” which is equivalent to 10mg of valsartan. F1 has more 10mg of drug release. Please clarify.

Response:

We thank the reviewer for pointing this out. In this study, we have calculated the drug recovery, showing the percentage more than 100%. However, this still met the requirement of drug content recovery set by the International Council for Harmonization (ICH), which was in the range of 95-105%. (Walfish, 2006). Accordingly, this could be the reason that the release amount was more than 10 mg.

Figure 2A shows 80% of valsartan was released in first two hours followed by remaining 20% in the next 6 hours. A clarification of this results will be helpful.

Response:

We thank the reviewer for the suggestion. As a result, we have included the explanations, as follows:

Specifically, it was found that in the first 2 hours, all formulations exhibited burst release, showing the release percentage of around 80%. Afterwards, the sustained release manner was shown in the following hours. The burst release occurred possibly due to the presence of VAL on the outer surface of the gel or in the gel surface layers voids.

We have now included this in the revised manuscript.

Section 3.8 – please also plot the occlusive data for control. Section 2.10 experimental section states that occlusive study was also performed on control. Also discuss how does the samples F1 through F4 compare against the control.

We thank the reviewer for the suggestion. As a result, we have included the results of the control and these explanations, as follows:

Additionally, it was also observed that there was no significant difference ($p > 0.05$) in the occlusivity values between all formulations with the control formulations,

We have now included this in the revised manuscript.

Section 3.9 – This section needs to be updated significantly. Data only for F1 is discussed. Please discuss in detail about comparison of each of the following:

For control, 0.5mm, 1.0mm and 1.5mm – F1 vs F2 vs F3 vs F4

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

For each formulation compare control vs 0.5mm vs 1.0mm vs 1.5mm.

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

A figure of dermaroller perforated skin will be helpful

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

Also, more acceptable way of plotting data is plot of “average cumulative percent drug release vs time”. This will also give release profile for each formulation.

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

Reviewer #4: 1. Thorough language revision and grammar check are needed.

Response:

We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. We have re-read the manuscript and have made a great effort to improve the English throughout.

2. Mention the abbreviation consistently throughout the manuscript. In the abstract, it is mentioned 'VALS,' but in the main content, it is mentioned 'VAL & VALS,' and it is also mentioned 'valsartan' in the main text.

Response:

We thank the reviewer for the suggestion. We have mentioned the abbreviation consistently in the revised manuscript.

3. It is mentioned that 'Higher amount of VALS released were observed as the concentration of Carbopol increase in the formulation.' Please explain this statement. Because in the same paragraph, it is noticed that the drug release is decreased due to the $(8.44 \pm 0.25 \text{ mg for F4})$ increased concentration of the carbopol.

Response:

We thank the reviewer for pointing this out. We do apologize for the confusion. Indeed, Carbopol concentration affected the release of VAL. A higher amount of VAL released was observed as the concentration of Carbopol decreased in the formulation. We have clarified this in the revised manuscript.

4. It is mentioned that the 'Higher occlusivity could increase skin hydration which facilitate drug penetration.' But it could take longer for the skin to reverse to its normal condition. So, it may irreversibly damage the skin. Please explain.

Response:

We thank the reviewer for the suggestion. As a result, we have included the explanations, as follows:

The occlusivity of formulations would affect the amount of drugs in the skin. Higher occlusivity could increase skin hydration which facilitates drug penetration. This is due to the reduction of corneocyte gaps (Ammar et al., 2016; Permana et al., 2020b). Additionally, it was also observed that there was no significant difference ($p > 0.05$) in the occlusivity values between all formulations with the control formulations. Accordingly, the addition of VAL did not alter the occlusivity of Carbomer-based gels. Several studies have shown the benefits of the occlusivity of topical preparation in the transdermal delivery system. The occlusivity of the preparations did not cause damage and irritation to the skin (Shrotriya et al., 2018, 2017; Wavikar and Vavia,

2013). Importantly, the values obtained in our study were found to be comparable with several previous reports. Accordingly, it might be hypothesized that the occlusivity properties of the gel did not possibly cause any damage in the skin.

We have now included this in the revised manuscript.

5. Occlusivity also influence the drug permeation. Is that considered in this study?

Response:

We thank the reviewer for the question. In this study, we found that there was no significant difference in the occlusivity in all formulations. Therefore, the viscosity and the combination with Dermalrollers® were considered as the main reason for the difference in the drug permeation profile.

6. Please follow the journal template, including the subsections' numbering.

Response:

We thank the reviewer for the suggestion. We have used the template of the journal and followed the guidelines required.

7. Include permeation coefficient in the results.

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

8. Describe the rationale and characteristics of the optimized formulation in detail.

Response:

We thank the reviewer for the suggestion. We have now included this in the revised manuscript.

9. In vitro hemolysis- is done only for the optimized formulation or all formulations?

Response:

We thank the reviewer for the question. In this study, we evaluated the hemolysis ability of VAL from F1 as the optimum formulation. We have now included this in the revised manuscript.

10. No skin irritation-requires strong justification, not just only pH.

Response:

We thank the reviewer for the suggestion. Indeed, the skin irritation study is required. Accordingly, we have rephrased the explanation to avoid the confusion. The developed VAL transdermal gels were considered safe and could not possibly cause irritation to the skin. To show the efficacy of this combination, further *in vivo* studies should be considered to assess the pharmacokinetic, pharmacodynamic and skin irritation studies.

REFERENCES:

- Al-Suwayeh, S.A., Taha, E.I., Al-Qahtani, F.M., Ahmed, M.O., Badran, M.M., 2014. Evaluation of skin permeation and analgesic activity effects of carbopol lornoxicam topical gels containing penetration enhancer. Sci. World J. 2014. <https://doi.org/10.1155/2014/127495>
- Alberti, I., Grenier, A., Kraus, H., Carrara, D.N., 2005. Pharmaceutical development and clinical effectiveness of a novel gel technology for transdermal drug delivery. Expert Opin. Drug Deliv. 2, 935–950. <https://doi.org/10.1517/17425247.2.5.935>

- Ammar, H.O., Ghorab, M.M., Mostafa, D.M., Ibrahim, E.S., 2016. Folic acid loaded lipid nanocarriers with promoted skin antiaging and antioxidant efficacy. *J. Drug Deliv. Sci. Technol.* 31, 72–82.
- Gorzelanny, C., Mess, C., Schneider, S.W., Huck, V., Brandner, J.M., 2020. Skin barriers in dermal drug delivery: Which barriers have to be overcome and how can we measure them? *Pharmaceutics* 12, 1–31. <https://doi.org/10.3390/pharmaceutics12070684>
- Lambers, H., Piessens, S., Bloem, A., Pronk, H., Finkel, P., 2006. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. *Int. J. Cosmet. Sci.* 28, 359–370. <https://doi.org/10.1111/j.1467-2494.2006.00344.x>
- Paudel, K.S., Milewski, M., Swadley, C.L., Brogden, N.K., Ghosh, P., Stinchcomb, A.L., 2010. Challenges and opportunities in dermal/transdermal delivery. *Ther. Deliv.* 1, 109–131. <https://doi.org/10.4155/tde.10.16>
- Permana, A.D., McCrudden, M.T.C., Donnelly, R.F., 2019. Enhanced intradermal delivery of nanosuspensions of antifilaria drugs using dissolving microneedles: A proof of concept study. *Pharmaceutics* 11, 346.
- Permana, A.D., Paredes, A.J., Volpe-Zanutto, F., Anjani, Q.K., Utomo, E., Donnelly, R.F., 2020a. Dissolving microneedle-mediated dermal delivery of itraconazole nanocrystals for improved treatment of cutaneous candidiasis. *Eur. J. Pharm. Biopharm.* 154, 50–61. <https://doi.org/10.1016/j.ejpb.2020.06.025>
- Permana, A.D., Utami, R.N., Courtenay, A.J., Manggau, M.A., Donnelly, R.F., Rahman, L., 2020b. 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.
- Proksch, E., 2018. pH in nature, humans and skin. *J. Dermatol.* 45, 1044–1052. <https://doi.org/10.1111/1346-8138.14489>
- Shrotriya, S., Ranpise, N., Satpute, P., Vidhate, B., 2018. Skin targeting of curcumin solid lipid nanoparticles-encapsulated topical gel for the treatment of pigmentation and irritant contact

dermatitis. *Artif. Cells, Nanomedicine Biotechnol.* 46, 1471–1482.

<https://doi.org/10.1080/21691401.2017.1373659>

Shrotriya, S.N., Ranpise, N.S., Vidhate, B. V., 2017. Skin targeting of resveratrol utilizing solid lipid nanoparticle-engrossed gel for chemically induced irritant contact dermatitis. *Drug Deliv. Transl. Res.* 7, 37–52. <https://doi.org/10.1007/s13346-016-0350-7>

Tripathi, P., Kumar, A., Jain, P.K., Patel, J.R., 2018. Carbomer gel bearing methotrexate loaded lipid nanocontainers shows improved topical delivery intended for effective management of psoriasis. *Int. J. Biol. Macromol.* 120, 1322–1334.
<https://doi.org/10.1016/j.ijbiomac.2018.08.136>

Walfish, S., 2006. A statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. *BioPharm Int.* 19, 28–36.

Wavikar, P., Vavia, P., 2013. Nanolipidgel for enhanced skin deposition and improved antifungal activity. *AAPS PharmSciTech* 14, 222–233. <https://doi.org/10.1208/s12249-012-9908-y>

Zhang, Y., Ng, W., Hu, J., Mussa, S.S., Ge, Y., Xu, H., 2018. Formulation and in vitro stability evaluation of ethosomal carbomer hydrogel for transdermal vaccine delivery. *Colloids Surfaces B Biointerfaces* 163, 184–191. <https://doi.org/10.1016/j.colsurfb.2017.12.031>

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ACCEPTED



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

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Stuart Cooper <em@editorialmanager.com>

Sat, Sep 3, 2022 at 5:09 AM

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Ref: JBS-D-22-00297R1

Improved transdermal delivery of valsartan using combinatorial approach of polymeric transdermal hydrogels and solid microneedles: An ex vivo proof of concept investigation
Journal of Biomaterials Science: Polymer Edition

Dear Dr. Permana,

I am pleased to tell you that your work has now been accepted for publication in Journal of Biomaterials Science: Polymer Edition.

It was accepted on Sep 02, 2022. You should receive the first proofs in approximately 4-6 weeks time.

Thank you for submitting your work to this journal.

With kind regards,

Stuart Cooper
Editor-in-Chief
Journal of Biomaterials Science: Polymer Edition

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