BUKTI SUBMIT MANUSKRIP

Universitas Hasanuddin

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

Submission received for Journal of Biomaterials Science, Polymer Edition (Submission ID: 226247195)

1 message

journalshelpdesk@taylorandfrancis.com <journalshelpdesk@taylorandfrancis.com> Sun, Sep 25, 2022 at 8:07 AM
To: andi.dian.permana@farmasi.unhas.ac.id

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Dear Andi Dian Permana,

Thank you for your submission.

Submission ID	**226247195**
Manuscript Title	**Polyvinyl Alcohol-based Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir**
Journal	**Journal of Biomaterials Science, Polymer Edition**

You can check the progress of your submission, and make any requested revisions, on the Author Portal.

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Kind Regards,
Journal of Biomaterials Science, Polymer Edition Editorial Office

BUKTI REVIEW DARI REVIEWERS

Universitas Hasanuddin

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

226247195 (Journal of Biomaterials Science: Polymer Edition) A revise decision has been made on your submission

2 messages

Stuart Cooper <em@editorialmanager.com> Sat, Nov 5, 2022 at 12:40 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-22-00407
226247195
Polyvinyl Alcohol-based Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir
Journal of Biomaterials Science: Polymer Edition

Dear Dr. Permana,

Your manuscript has been reviewed by four 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 Dec 04, 2022.

To submit a revision, go to https://rp.tandfonline.com/submission/flow?submissionId=226247195&step=1. 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.

If you have any questions or technical issues, please contact the journal's editorial office at SP-Ingest@journals.tandf.co.uk.

Yours sincerely,

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

Comments from the Editors and Reviewers:

Reviewer #1: This is a fairly nice piece of work that will add to knowledge in the field. The study has clearly been well-planned, carefully executed and data meticulously analysed. Statistical treatment of data is appropriate and the conclusions drawn are sensible.

This work will add to the ever-growing body of evidence on the effectiveness of microneedle systems for drug delivery. The paper is likely to be widely read and, in due course, cited. Having said all that, I'm not so sure about originality of the concept here. One particular Group has pioneered hydrogel-forming microneedles. Indeed, the corresponding author here was, I think, at one time a member of that Group. What differentiates the present study from work in his previous team now that he has his own Group?

The authors should consider translation of this technology. Will regulatory bodies demand sterility of microneedle patches? What manufacturing and distribution challenges will this present? What about storage stability? How would this device be reproducibly inserted by patients or their carer? Would an applicator be used or would the microneedles be inserted by hand? If inserted by hand, how would the patient or carer know for sure they had pressed the microneedles in to the skin with sufficient force? How would they obtain feedback? How long would the microneedles need to be left in the skin for effective delivery? Would this be practical, given the rapid delivery of medicines when taken orally? What about disposal? How would this be done safely and securely in resource-poor settings?

These microneedles may deposit uncrosslinked polymer in skin. How quickly would it biodegrade? Would it accumulate in skin or the draining lymph node local to the site of application? How would it be excreted? These are

important translational considerations, as is scaled-up manufacture. Would the described production method really be suitable for manufacture of the numbers of patches required for a commercialised product?

The authors need to show the set-up with the liquid reservoirs, rather than just referring to previous work

There are numerous typos and omissions - A thorough re-read is necessary to improve readability and also make it clear how experiments were done

Check the references for accuracy and consistency. Reference 5, as one example, mixes up surname and first name of the lead author

Reviewer #2: The manuscript presents serious gaps. There is no rationale for developing a microneedle for albendazole transdermal delivery since the site of action of this anthelmintic drug is local, the gastrointestinal tract. Concerning the methodology, the article is confusing. Microscopic characterization is essential in MN, especially in this case, that present a "liquid reservoir". Microscopic evaluation of the microneedles was not described in the methodology. Images of fig. 4 should be improved, it is not possible to evaluate the system in these images. The skin studies are also not clear. The source of skin is not disclosed and raises concerns about the necessity of ethics committee evaluation. The cumulative amount of ABZ permeated through the skin was impressive and the method of analysis is not detailed (UV/Vis spectrophotometry does not seem a method with good specificity for such assay). In my point of view, the manuscript must be rejected and undergo major revision before resubmission. Albendazole should be classified as a model drug and for this, more physicochemical characteristics should be included and discussed.

Reviewer #4: Manuscript ID: JBS-D-22-00407
Title: Polyvinyl Alcohol-based Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir

This manuscript from Permana's group is reporting a good work about transdermal delivery of albendazole. Orally administered ABZ tends to have limited efficacy due to its poor solubility. In order to enhance its delivery to therapeutic target, polyvinyl alcoholbased hydrogel-forming microneedles (HFMs) was developed. HFMs can effectively deliver drugs loaded in the reservoir through transdermal route with fewer side effects and longer therapeutic duration. In addition, to enhance ABZ's solubility, drug can be loaded as liquid reservoir using water-miscible solvents, which will effectively enhance the solubility of ABZ, resulting in higher bioavailability. All the results suggested this innovation has a huge potential to overcome the limitations of oral ABZ's delivery and potentially enhance its therapeutic effect through the transdermal route. This study is an interesting work. I would like to recommend the publication of this work after revision. However, the authors should solve the following issues.
The issues in the manuscript are listed as below:
1. Introduction, the design of microneedles for transdermal delivery had been investigated wildly in the field of biomedical, and some relative works must be cited to enrich the background of this paper. Please see references (Asian Journal of Pharmaceutical Sciences 2022, 17: 70-86; Actively Separated Microneedle Patch for Sustained-Release of Growth Hormone to Treat Growth Hormone Deficiency, Acta Pharmaceutica Sinica B 2022 https://doi.org/10.1016/j.apsb.2022.04.015).
2. The innovation of this manuscript could be presented in detail in the introduction.
3. The tables must be shown as three-line table form.
4. For Figure 3A, the error bar is missing. The authors should add it in the figure.
5. Overall the paper is easily read, but there are still some minor problems in language and spelling. A throughout checking is recommended.
6. The introduction section can be further improved by the following references.
Bioactive Materials 2022, 17: 49-70; Acta Pharmaceutica Sinica B 2021, 11: 2937-2944； Smart Materials in Medicine, 2021.https://doi.org/10.1016/j.smaim.2021.12.005.

Reviewer #5: Rectify the following issues:
1) Title- Polyvinyl Alcohol-based Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir -- correct it to shorter one.
2) Explain more about the FTIR molecular interaction.
3) Which animal skin was used ?
4) there is no importance of skin integrity test. 24hr is impractical.

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id> Sat, Nov 5, 2022 at 11:08 AM
To: anugerahyaumil@gmail.com

[Quoted text hidden]

BUKTI SUBMIT HASIL REVIEW

Universitas Hasanuddin

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

Revised submission received for Journal of Biomaterials Science, Polymer Edition (Submission ID: 226247195.R1)

1 message

journalshelpdesk@taylorandfrancis.com <journalshelpdesk@taylorandfrancis.com> Mon, Nov 14, 2022 at 9:47 PM
To: andi.dian.permana@farmasi.unhas.ac.id

Taylor & Francis
Taylor & Francis Group

Dear Andi Dian Permana,

Thank you for submitting your revised manuscript.

Submission ID	**226247195**
Manuscript Title	**Development of Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir**
Journal	**Journal of Biomaterials Science, Polymer Edition**

You can check the progress of your submission, and make any requested revisions, on the Author Portal.

Thank you for submitting your work to our journal.
If you have any queries, please get in touch with journalshelpdesk@taylorandfrancis.com.

Kind Regards,
Journal of Biomaterials Science, Polymer Edition Editorial Office

Comments from the Editors and Reviewers:

Reviewer #1:

Q1: This is a fairly nice piece of work that will add to knowledge in the field. The study has clearly been well-planned, carefully executed and data meticulously analysed. Statistical treatment of data is appropriate and the conclusions drawn are sensible. This work will add to the ever-growing body of evidence on the effectiveness of microneedle systems for drug delivery. The paper is likely to be widely read and, in due course, cited. Having said all that, I'm not so sure about originality of the concept here. One particular Group has pioneered hydrogel-forming microneedles. Indeed, the corresponding author here was, I think, at one time a member of that Group. What differentiates the present study from work in his previous team now that he has his own Group?

A1: We thank for the reviewer for taking time to giving us all the great reviews and comments. The research about hydrogel-forming microneedles (HFMs) has been done by some research groups. However, in this study, the transdermal delivery of Albendazole (ABZ) was done through a different approach. We successfully developed polyvinyl alcohol-based HFMs along with ABZ that loaded as liquid reservoir using water-miscible solvent to enhanced its solubility. Various evaluations and characteristics have been done and all the result suggested this innovation has a huge potential to overcome the limitations of other conventional delivery of ABZ.

Q2: The authors should consider translation of this technology. Will regulatory bodies demand sterility of microneedle patches?

Q2: We are thankful to the reviewer for the question. We have been added the explanation regarding about the sterility issue in the Section 1 (Introduction) of our revised manuscript as follows:

Therefore, another thing that needs to be consider is the sterilization issue of the HFMs. Previous study [1] shown that repeated application of MNs into the skin did not cause any decrease on the skin barrier function. The administration of MNs also did not cause the microorganism to penetrate through the skin. Moreover, it has been reported that the use of polymer-based MNs, such as HFMs, would not stimulate the humoral immune system and consider to be safe even after repeated applications. It could be concluded that the risk of infection was consider low in the administration of HFMs. However, more research needs to be conducted to ensure the effectiveness, therapeutic safety, and the sterilization issues of HFMs [1].

Q3: What manufacturing and distribution challenges will this present?

A3: We thank to the reviewer for the question. We have been added the explanation about manufacturing and distribution challenges in the Section 1 (Introduction), as follows:

Moreover, the most challenging problem on HFMs fabrication was also the availability of low-cost manufacturing methods [2]. In this study, we used various materials, such as PVA, with several beneficial advantages and consider to be cheaper than other polymers to reduce the cost manufacturing. Regarding about the manufacturing and distribution challenges of MNs, many clinical trials and advancement of MNs technologies proved that MNs could be potentially use for commercial. In addition, there are some of MNs devices which already reached the commercial market for both diagnostic and therapeutic applications [3].

Q4: What about storage stability?

A4: We thank the reviewer for the question. We have included the explanation about this in the Section 3.3 and Section 3.5 of our revised manuscript, as follow:

Section 3.3:

A water vapor transmission (WVT) test was conducted to determine the hydrogel's permeability characteristics, showing the quantity of moisture transmitted through the unit area of the hydrogel in unit time. After seven days, the WVT rate turned out to be 0.53 ± 0.04 mg.cm/cm2 at 24 h, indicating that the H1 formulation was permeable to water vapor, probably due to the PVP content which is one of the hygroscopic polymers. However, this WVT rate is still considered low and potentially indicates the hydrogel's long-term stability [4][5].

Section 3.5:

Moisture absorption indicated hydrogel's affinity to uptake the external water molecules. In addition, the higher moisture absorption rate may affect the functionality of MNs under storage condition. Based on the results shown in Fig.3.C, the higher RH values directly affect the increase of moisture absorption ability of HFMs H1. After 14 days of testing, all formulas had a moisture absorption percentage of <10%. It is probably due to the existence of PVP in a hydrogel formulation. PVP is a unique polymer that has a strong moisture absorption ability due to its hygroscopic characteristic. In this case, the PVP used in the formulation will directly affect the moisture absorption ability of the hydrogel [6]. Furthermore, the changes in RH can also affect the ability of the hydrogel to absorb the external water. Generally, the higher RH in the environment will cause a higher value of moisture absorption [7]. However, the moisture absorption of HFMs H1

was still consider as low compared to other MNs formula containing PVP that gained up to 14-16% of moisture absorption [4].

Q5: How would this device be reproducibly inserted by patients or their career? Would an applicator be used or would the microneedles be inserted by hand? If inserted by hand, how would the patient or career know for sure they had pressed the microneedles in to the skin with sufficient force? How would they obtain feedback?

A5: We thank the reviewer for the question. We have been added the information about this in the manuscript, as follows:

Unlike common conventional transdermal drug delivery systems, the combination of HFMs and liquid reservoir have high potential to reduce the risk of cross-contamination, which is often appearing during repeated application of other conventional drug administration [8]. After being inserted through the skin, the HFMs will absorb the interstitial fluid and swell, then the ABZ loaded in the reservoir will permeate into the cell through passive diffusion [9]. With that being said, the use of the HFMs and its reservoir are not designed to be use reproducibly, resulting in low possibility of cross-contamination. In addition, this innovation also tends to increase the convenience of patient due to its ease of application. Considering the acceptance and patient convenience, the HFMs is preferred to be inserted by hand. Previous study [10] shows that insertion of MNs by hand tend to be easier and resulting in better patient compliance. However, in order to penetrate through the stratum corneum, the MNs need to be pressed with certain force, so the use of a pressure-indicating sensor can be an alternative option. Based on previous study, the minimum pressure required for the application of 1 cm^2 of MNs to be able to penetrate to the stratum corneum is 32 N/cm2. The pressure-indicating sensor will change its colour when the applied forces reach 30 N and even be more concentrated if given a greater force [1].

Q6: How long would the microneedles need to be left in the skin for effective delivery?

A6: We thank the reviewer for the question. Based on the *ex vivo* permeation test that have been conducted in this study, after 24 hours of application, the cumulative amount of ABZ permeated into the skin reached up to 971.23 ± 11.77 $\mu g/cm^2$. However, to ensure the duration of effective delivery of this innovation, pharmacokinetic and toxicity study should be conducted.

Q7: Would this be practical, given the rapid delivery of medicines when taken orally?

A7: We thank for the reviewer for the question. Based on various studies, the use of microneedles is more practical than oral administration of several drugs, since the use of microneedle will be avoiding first pass metabolism. Furthermore, ABZ is known to

has poor water solubility, resulting in low bioavailability when administrated orally. As a result, the oral preparation of ABZ requires higher doses and potentially causing more side effects [11][12]. Meanwhile, drug administration using microneedle can provide several advantages, such as preventing the occurrence of first pass metabolism and usually require only low doses to achieve a therapeutic effect compared to oral preparations [13] [14]. We also include this explanation in the revised manuscript, as follow:

As anthelmintic agent, ABZ is commonly found in oral preparations [15]. However, according to Biopharmaceutical Classification System (BCS), ABZ had been categorized as borderline BCS class II/IV, having low solubility and entirely insoluble in water and most organic solvents [16]. Previous studies [11][12] have shown that ABZ is a weak base whose solubility depends on the solvent's pH. After being orally administrated, its solubility will drop sharply in gastric (pH 1.4) and intestinal fluids (pH 6.5), resulting in poor bioavailability and limited drug efficacy. As a result, ABZ is often delivered as high-dose oral preparations, resulting in more adverse effects, especially in patients with poor liver function. Recorded between 2006 and 2015, according to The Korean Institute of Drug Safety & Risk Management data, there were 256 probable or possible adverse effects caused by ABZ in oral preparation [12]. Therefore, oral administration of ABZ has become a significant burden due to its severe adverse effects.

Q8: What about disposal? How would this be done safely and securely in resource-poor settings?

A8: We thank the reviewer for giving the question for clarification. Based on the question above, we have been added explanation about the disposal of microneedles in the Section 1 (Introduction) of our revised manuscript, as follows:

In terms of material, a biodegradable polymer was added as the base of HFMs. Polyvinyl alcohol (PVA) is one of the most used polymers in biomedical applications systems and devices due to its water solubility, low toxicity, biocompatibility, excellent physical properties, and known to be able to overcome various problems associated with safety and disposal due to its biodegradable characteristic [17][18][19].

Q9: These microneedles may deposit uncrosslinked polymer in skin. How quickly would it biodegrade? Would it accumulate in skin or the draining lymph node local to the site of application?

A9: We are thankful for the reviewer for the question. We have been added the explanation about this in Section 1 (Introduction) of our revised manuscript:

The previous study [20] explained that PVA would deposit under the skin after microneedle administration. Additionally, it was found that after six days, the deposition concentration of PVA in the insertion sites gradually decreased and disappeared entirely from the skin after 28 days of application. However, there were never recorded cases associated with the accumulation of PVA in the skin or drained the lymph nodes at the application site [21].

Q10: How would it be excreted?

A10: We thank the reviewer for the question. We have explained about this in Section 1 (introduction) of our revised manuscript, as follows:

Unlike common conventional transdermal drug delivery systems, the combination of HFMs and liquid reservoir have high potential to reduce the risk of cross-contamination, which is often appearing during repeated application of other conventional drug administration [8]. After being inserted through the skin, the HFMs will absorb the interstitial fluid and swell, then the ABZ loaded in the reservoir will permeate into the cell through passive diffusion [9]. Then, the HFMs will directly be removed from the skin and the ABZ will be distributed in the body, entered the liver, where ABZ is metabolized, assisted by recombinant cytochrome p450 enzymes [22]. Most of ABZ will be excreted through bile and a small portion will be excreted through urine.

Q11: These are important translational considerations, as is scaled-up manufacture. Would the described production method really be suitable for manufacture of the numbers of patches required for a commercialized product?

A11: We are thankful to the reviewer for the question. Based on previous study, various manufacturing methods are widely introduced, including the MNs and MN molds manufacture, which allows for the cost-effective production for commercialized use [3]. In this study, we used micro-molding method and filled the micro holes by utilizing centrifugation to remove the excess bubbles. However, we are also considering that this method is not suitable for scaled-up manufacture. It is so essential to reconsider the fabrication method and find an ideal method to produce microneedles in large quantities.

Q12: The authors need to show the set-up with the liquid reservoirs, rather than just referring to previous work.

A12: We are thankful to the reviewer for the question. We have been added the set-up animation of the MNs incorporating with the liquid reservoir in as showed in Figure 1. in the revised manuscript.

Q13: There are numerous typos and omissions - A thorough re-read is necessary to improve readability and also make it clear how experiments were done. Check the references for accuracy and consistency. Reference 5, as one example, mixes up surname and first name of the lead author

A13: We are very thankful to the reviewer for pointing this out. We have been checked and done the proofreading process to fixed the numerous typos and omissions.

Reviewer #2:

Q1: The manuscript presents serious gaps. There is no rationale for developing a microneedle for albendazole transdermal delivery since the site of action of this anthelmintic drug is local, the gastrointestinal tract.

A1: We are thankful to the reviewer for taking the time to give reviews and comments for our future improvement. As mentioned in Introduction (Section 1), Albendazole (ABZ) is not only used in the treatment of intestinal or local infections, but it is also widely known as first-line treatment in various systemic infectious, such as echinococcal infections [22]. However, ABZ has low solubility, resulting in poor bioavailability and limited drug efficacy when administered as oral preparations [16]. Furthermore, there are 256 probable adverse effects caused by ABZ in oral preparation [12]. In order to overcome the limitation of oral preparation of ABZ, developing the transdermal drug delivery of ABZ can be an appropriate alternative. Considering the existence of *stratum corneum* in the skin that may limit several drug's permeation, preparing pharmaceutical dosage forms through microneedles (MNs) would be an optimum option, since MNs can penetrate through the *stratum corneum* and allow drug to enter the systemic circulation [23].

Q2: Concerning the methodology, the article is confusing. Microscopic characterization is essential in MN, especially in this case, that present a "liquid reservoir". Microscopic evaluation of the microneedles was not described in the methodology.

A2: We thank to the reviewer for pointing this out. We have added the description of microscopic evaluation of the microneedles in the Section 2.10 in the revised manuscript as follows:

The penetration test was carried out using eight layers of Parafilm® (≈1 mm; 126 µm/layer). HFMs were attached and lowered onto the eight sheets of Parafilm® and given a load until it met the required force of 32 N, exerted and held for 30 seconds. Following the penetration test, the Parafilm® sheets were unfolded. Then, the number of holes formed in each layer of Parafilm® was counted [10][24]. A mechanical strength test was carried out using a similar method to the penetration test. The HFMs were placed on eight layers of Parafilm® and given a load of 32 N for 30 seconds. The microneedle height before and after the mechanical strength test was compared by using the optical microscope (Olympus® CX23 LED) and measured using Image J (National Instrument of Health, USA) [25].

Q3: Images of fig. 4 should be improved, it is not possible to evaluate the system in these images.

A3: We are thankful for the reviewer for the helpful suggestion. We have improved the Figure 4. as showed in the revised manuscript.

Q4: The skin studies are also not clear. The source of skin is not disclosed and raises concerns about the necessity of ethics committee evaluation.

A4: We are grateful to the reviewer for pointing this out. We have added a clear explanation regarding about the skin preparation for the study, including the source of skin and ethics committee explanation in the Section 2.15 of the revised manuscript, as follows:

All the animal used in this study were treating based on the protocol approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Hasanuddin University, Indonesia. One day before the *ex vivo* permeation study, the skin hair of a euthanized rat was shaved. The abdominal skin was cut out and the fat layer was removed from the skin. Then, the skin was washed using PBS solution (pH 7.4) repeatedly and the skin thickness was measured by using a digital calliper (Taffware® LCD-XY, Indonesia). Afterwards, the skin was wrapped in aluminium foil and placed in the freezer (-20^{o}C) for further testing.

Q5: The cumulative amount of ABZ permeated through the skin was impressive and the method of analysis is not detailed (UV/Vis spectrophotometry does not seem a method with good specificity for such assay).

A5: We are very thankful to the reviewer for giving us the helpful comment for clarification. UV/Vis spectrophotometry method used in this study has been validated, consisting of linearity, specificity, accuracy, precision, detection limit and quantification limit in accordance to the International Conference Harmonization (ICH) guidelines. Based on the results, there was the linear correlation between the absorbance and the concentration of ABZ with the coefficient correlation value was 0.9995, and considering to be accepted as its value was close to 1 [26]. Furthermore, the LOD and LOQ values were 0.4845 and 1.4682, respectively, which indicated the sensitivity of the validated method. The relative standar deviation (RSD) and the relative error (RE) values after intra- and inter-day precision were within the required limits of ±15% [27] at all the concentration levels. These data showed that the method developed has an adequate accuracy and precision to be used for further analysis.

Q5: In my point of view, the manuscript must be rejected and undergo major revision before resubmission. Albendazole should be classified as a model drug and for this, more physicochemical characteristics should be included and discussed.

A5: We are very thankful to the reviewer for giving us the helpful comments and suggestions. We have revised our manuscript based on the recommendations and suggestions that given by the Reviewer, so that we believe that our revised manuscript is now substantially more improved and worth to be reconsidered.

We have been added the FTIR interpretation of ABZ in Section 3.7 as the physicochemical characterisation of the model drug, as follows:

As shown in **Fig.3.E**, ABZ spectra showed the N-H stretching in the 3350-3310 cm^{-1} and the peak at 3332,99 cm^{-1}. It was also noticed that the valence vibrations of the aromatic were found in the range of 1650-2000 cm^{-1}. Around 1500-1700 cm^{-1}, the valence vibration of the group carboxyl C=O from ester group was appeared. The valence vibrations of the C-O stretching were identified in the 1250-1310 cm^{-1} from group ester and the peak showed at 1192,01 cm^{-1}.

Reviewer #3: Manuscript ID: JBS-D-22-00407
Title: Polyvinyl Alcohol-based Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir

This manuscript from Permana's group is reporting a good work about transdermal delivery of albendazole. Orally administered ABZ tends to have limited efficacy due to its poor solubility. In order to enhance its delivery to therapeutic target, polyvinyl alcohol based hydrogel-forming microneedles (HFMs) was developed. HFMs can effectively deliver drugs loaded in the reservoir through transdermal route with fewer side effects and longer therapeutic duration. In addition, to enhance ABZ's solubility, drug can be loaded as liquid reservoir using water-miscible solvents, which will effectively enhance the solubility of ABZ, resulting in higher bioavailability. All the results suggested this innovation has a huge potential to overcome the limitations of oral ABZ's delivery and potentially enhance its therapeutic effect through the transdermal route. This study is an interesting work. I would like to recommend the publication of this work after revision. However, the authors should solve the following issues.

The issues in the manuscript are listed as below:
Q1: Introduction, the design of microneedles for transdermal delivery had been investigated wildly in the field of biomedical, and some relative works must be cited to enrich the background of this paper. Please see references (Asian Journal of Pharmaceutical Sciences 2022, 17: 70-86; Actively Separated Microneedle Patch for Sustained-Release of Growth Hormone to Treat Growth Hormone Deficiency, Acta Pharmaceutica Sinica B 2022 https://doi.org/10.1016/j.apsb.2022.04.015).

A1: We are very grateful for the helpful suggestion. We have enriched the background of this paper and added more explanations in Intoduction (Section 1) of our revised manuscript.

Q2: The innovation of this manuscript could be presented in detail in the introduction.

A3: We are thankful the reviewer for the helpful suggestion. We have been added more explanations regarding about the innovation in the introduction (Section 1) of our revised manuscript.

Q3: The tables must be shown as three-line table form.

A3: We are thankful for the reviewer for pointing this out. We have been fixed all the tables and arranged them in three-line table form as shown in our revised manuscript.

Q4: For Figure 3A, the error bar is missing. The authors should add it in the figure.

A3: We are grateful for the reviewer for pointing this out. We have been fixed the figure and added the error bars.

Q5: Overall the paper is easily read, but there are still some minor problems in language and spelling. A throughout checking is recommended.

A5: We are very thankful to the reviewer for pointing this out. We have been checked and done the proofreading process to fixed the minor problems.

Q6: The introduction section can be further improved by the following references. Bioactive Materials 2022, 17: 49-70; Acta Pharmaceutica Sinica B 2021, 11: 2937-2944 ; Smart Materials in Medicine, 2021.https://doi.org/10.1016/j.smaim.2021.12.005.

A6: We are very thankful to the reviewer for the suggestion. We have been improved the introduction by the recommended reference.

Reviewer #4: Rectify the following issues:

Q1: Title- Polyvinyl Alcohol-based Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir -- correct it to shorter one.

A1: We thank the reviewer for the suggestion. We have been corrected the title and made it shorter, as follow:

"Development of Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir"

Q2: Explain more about the FTIR molecular interaction.

A2: We are thankful to the reviewer for the suggestion. We have added more explanation about the FTIR molecular interaction, as follows in the Section 3.7 of our newest revised manuscript:

As shown in Fig.3.F, the intermolecular interactions of tartaric acid molecules with PVA polymer chains are shown by several inter- and intramolecular cross-links formed. The position of the band shifted and the absorbance of this band increased significantly with the increasing of tartaric acid content, indicating an increase in cross-link density in the structure. The absorbance of the carbonyl group increased with increasing TA concentration due to the esterification process. The cross-linking of the PVA chains results in the reduction of the hydroxyl groups of the PVA chains and the formation of new cross-linked ester bonds with the carbonyl strain [28].

Q3: Which animal skin was used?

A3: The skin used in this study is the abdominal skin of rat. All the animal study is based on the protocol approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Hasanuddin University, Indonesia. We have added an explanation regarding about this in the Section 2.15 of the revised manuscript, as follows:

All the animal used in this study were treating based on the protocol approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Hasanuddin University, Indonesia. One day before the *ex vivo* permeation study, the skin hair of a euthanized rat was shaved. The abdominal skin was cut out and the fat layer was removed from the skin. Then, the skin was washed using PBS solution (pH 7.4) repeatedly and the skin thickness was measured by using a digital calliper (Taffware® LCD-XY, Indonesia). Afterwards, the skin was wrapped in aluminium foil and placed in the freezer (-20^{o}C) for further testing.

Q4: There is no importance of skin integrity test. 24hr is impractical.

A4: We are thankful to the reviewer for pointing this thing out for clarification. As mentioned in the Section 2.16, the skin integrity test is essential to investigate the effect of HFMs application after a certain period of time [29]. This test is conducted in purpose to determine the effect of HFMs application to the skin integrity. If there were no changes found in skin integrity after 24 hours of HFMs application, it can be concluded that the HFMs is consider to be safe enough for the patient, at least for 24 hours of continuous use.

References:

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Development of Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir

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Development of Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir

Albendazole (ABZ) is an anthelmintic agent from the benzimidazole group, known as the broad-spectrum antiparasitic drug. ABZ is commonly used to treat human intestinal and systemic infections. Orally administered ABZ tends to have limited efficacy due to its poor solubility. In order to enhance its delivery to the therapeutic target, polyvinyl alcohol-based hydrogel-forming microneedles (HFMs) was developed. HFMs can effectively deliver drugs loaded in the reservoir through the transdermal route with fewer side effects and longer therapeutic duration. In addition, to enhance ABZ's solubility, the drug can be loaded as a liquid reservoir using water-miscible solvents, which will effectively enhance the solubility of ABZ, resulting in higher bioavailability. In this study, HFMs was successfully developed with high swelling abilities, more than 400%. Moreover, the penetration result showed HFMs could penetrate up to 63% into the skin with only a 7.14% of height decrease. The skin integrity test also showed HFMs permeation into the skin, causing no changes in skin integrity after 24 hours of application. Incorporated with the liquid reservoir, the *ex vivo* permeation test showed that the cumulative amount of ABZ permeated through the skin was about 971.23 ± 11.77 µg/cm^2. In conclusion, this innovation has a huge potential to overcome the limitations of ABZ in oral preparations and potentially enhance its therapeutic effect through the transdermal route.

Keywords: Albendazole, polyvinyl alcohol, hydrogel-forming microneedles, liquid reservoir, transdermal delivery

1. Introduction

Albendazole (ABZ) is a broad-spectrum antiparasitic agent used as the first-line treatment for both intestinal and systemic infections in humans. In the treatment of various systemic infections, ABZ is also known to be widely used as a first-line treatment of echinococcal infections [1]. As anthelmintic agent, ABZ is commonly found in oral preparations [2]. However, according to Biopharmaceutical Classification System (BCS), ABZ had been categorized as borderline BCS class II/IV, having low

solubility and being entirely insoluble in water and most organic solvents [3]. Previous studies [4][5] have shown that ABZ is a weak base whose solubility depends on the solvent's pH. After being orally administrated, its solubility will drop sharply in gastric (pH 1.4) and intestinal fluids (pH 6.5), resulting in poor bioavailability and limited drug efficacy. As a result, ABZ is often delivered as high-dose oral preparations, resulting in more adverse effects, especially in patients with poor liver function. Recorded between 2006 and 2015, according to The Korean Institute of Drug Safety & Risk Management data, there were 256 probable or possible adverse effects caused by ABZ in oral preparation [5]. Therefore, oral administration of ABZ has become a significant burden due to its severe adverse effects.

Developing another formulation with an improved drug administration route is essential to enhance the delivery of ABZ into infectious areas in the body. The skin, the human body's largest organ, provides a large area for drug absorption. The transdermal route has long been extensively studied and has been praised foremostly for its ease of application. The transdermal drug delivery is a potential drug administration route that offers some promising benefits, including the ability to rapidly deliver drugs to the target with the avoidance of first-pass metabolism [4]. Thus, during this transdermal route, the drug can delivered to the desired selective target by suppressing several side effects [6]. Considering the existence of *stratum corneum* that may limit several drugs' permeation, preparing pharmaceutical dosage forms through microneedles (MNs) would be an optimum option [7].

MNs are minimally-invasive arrays consisting of numerous micron-sized needles assembled on a baseplate, which can penetrate through the stratum corneum and enable drug penetration [8]. Among other drug administrations, MNs typically only require a low dose to achieve the therapeutic effect [9][10]. Furthermore, such MNs are

painless and may be more comfortable for the patient during therapy. In terms of storage stability, MNs is widely known to be able to maintain its stability even without specialized storage condition. Previous study [11] showed that microneedles had no significant loss of the drugs during storage at 25ºC for up to one year and exposure to 60ºC for four months.

Currently, MNs are widely developed and have multiple types with different advantages. One of the MNs types used recently due to their vast benefits is hydrogel-forming microneedles (HFMs). HFMs can offer transdermal drug delivery by readily uptake the interstitial fluid in the human body. HFMs also have a higher drug loading capacity than conventional MNs and offer a tunable drug release rate. Either by incorporating drugs into its polymeric structure or loading drugs into a reservoir, HFMs can effectively be used for transdermal drug delivery. More importantly, HFMs can function as a rate-controlling membrane, allowing sustained drug delivery [12].

In terms of material, a biodegradable polymer was added as the base of HFMs. Polyvinyl alcohol (PVA) is one of the most used polymers in biomedical applications systems and devices due to its water solubility, low toxicity, biocompatibility, excellent physical properties, and known to be able to overcome various problems associated with safety and disposal due to its biodegradable characteristic [13][14][15]. The previous study [16] explained that PVA would deposit under the skin after microneedle administration. Additionally, it was found that after six days, the deposition concentration of PVA in the insertion sites gradually decreased and disappeared entirely from the skin after 28 days of application. However, there were never recorded cases associated with the accumulation of PVA in the skin or drained the lymph nodes at the application site [17]. Due to its vast advantages, PVA was chosen as a suitable HFMs-base used in this study.

In development of HFMs as potential drug administration, considering the translation of this technology is quite critical. After many years of studies and researches, there are many significant developments of MNs technology commercialization. Currently, there are some devices containing MNs by both the academic and industrial researchers alike which are waiting for FDA approval [18]. Therefore, another thing that needs to be consider is the sterilization issue of the HFMs. Previous study [19] shown that repeated application of MNs into the skin did not cause any decrease on the skin barrier function. The administration of MNs also did not cause the microorganism to penetrate through the skin. Moreover, it has been reported that the use of polymer-based MNs, such as HFMs, would not stimulate the humoral immune system and consider to be safe even after repeated applications. It could be concluded that the risk of infection was consider low in the administration of HFMs. However, more research needs to be conducted to ensure the effectiveness, therapeutic safety, and the sterilization issues of HFMs [19].

Moreover, the most challenging problem on HFMs fabrication was also the availability of low-cost manufacturing methods [20]. In this study, we used various materials, such as PVA, with several beneficial advantages and consider to be cheaper than other polymers to reduce the cost manufacturing. Regarding about the manufacturing and distribution challenges of MNs, many clinical trials and advancement of MNs technologies proved that MNs could be potentially use for commercial. In addition, there are some of MNs devices which already reached the commercial market for both diagnostic and therapeutic applications [21].

Incorporating with the HFMs, drugs can be loaded into the reservoir-base and permeate into the skin through the MNs [12]. In this study, ABZ was formulated into a liquid reservoir using water-miscible solvents to enhance its solubility. The liquid

reservoirs were formulated in three different formulations using the combination of PEG 400 and PEG 600. The previous study [22] shows that using PEG 400 and co-solvent could rapidly increase the solubility of ABZ. Adding co-solvents will enhance the drug solubility by reducing the polarity of the entire system [4]. Thus, the formulation of ABZ into a liquid reservoir could be considered as an appropriate option.

Unlike common conventional transdermal drug delivery systems, the combination of HFMs and liquid reservoir have high potential to reduce the risk of cross-contamination, which is often appearing during repeated application of other conventional drug administration [23]. After being inserted through the skin, the HFMs will absorb the interstitial fluid and swell, then the ABZ loaded in the reservoir will permeate into the cell through passive diffusion [12]. Then, the HFMs will directly be removed from the skin and the ABZ will be distributed in the body, entered the liver, where ABZ is metabolized, assisted by recombinant cytochrome p450 enzymes [1]. Most of ABZ will be excreted through bile and a small portion will be excreted through urine. With that being said, the use of the HFMs and its reservoir are not designed to be use reproducibly, resulting in low possibility of cross-contamination. In addition, this innovation also tends to increase the convenience of patient due to its ease of application. Considering the acceptance and patient convenience, the HFMs is preferred to be inserted by hand. Previous study [24] shows that insertion of MNs by hand tend to be easier and resulting in better patient compliance. However, in order to penetrate through the stratum corneum, the MNs need to be pressed with certain force, so the use of a pressure-indicating sensor can be an alternative option. Based on previous study, the minimum pressure required for the application of 1 cm^2 of MNs to be able to penetrate to the stratum corneum is 32 N/cm2. The pressure-indicating sensor will

change its colour when the applied forces reach 30 N and even be more concentrated if given a greater force [19].

Generally, this study aims to develop a novel transdermal delivery of ABZ with rapid and higher permeation across the stratum corneum using HFMs incorporated with a liquid reservoir.

2. Materials and Methods

2.1 Materials

Albendazole (purity, ≥98+%) of analytical grade was purchased from Alfa Aesar (Lancashire, U.K). PVA (typically average M_w = 72.000 g/mol), sodium alginate (SA) (medium viscosity), and carbomer 980 were purchased from Sigma-Aldrich Pte Ltd. (Singapore, Singapore). Polyvinylpyrrolidone (PVP) K-30 was purchased from Fadjar Kimia (Bogor, Indonesia). Poly(ethylene glycol) (PEG 600 Da), tartaric acid, absolute ethanol, glycerin, and propylene glycol were purchased from Merck Schuchardt OHG (Hohenbrunn, Germany). PEG 400 and Tween80® were purchased from idCHEM Co., Ltd. (Gyeonggi, South Korea). Tablet phosphate-buffered saline (PBS) used in the swelling and dissolution study was purchased from Dulbecco A Oxoid Ltd. (Hampshire, United Kingdom). Silicon template, ST-08 10x10 array, was purchased from Micropoint Technologies P.T.E. Ltd. All other chemicals and materials used in this study were of analytical grade.

2.2 Fabrication of chemically cross-linked hydrogels

Chemically cross-linked hydrogels were fabricated from an aqueous polymeric blend containing PVA, PVP K30, carbomer 980, and tartaric acid. Different proportions of each compound were showed in **Table 1**. Based on previous studies [25], the use of PVA and PVP in the hydrogel formulation is known to produce MNs with optimal

characteristics. In addition, carbomers can build hydrogen bonds between the carbomer's carboxyl group and the PVA's hydroxyl group, resulting in a stronger hydrogel bond. Carbomer can also increase the viscosity of the formula due to the presence of hydrogen bonds which can produce MNs with better mechanical strength and swelling ability [26]. As a cross-linking agent, tartaric acid containing carboxyl and hydroxyl groups which can build an active site in the non-covalent interaction bond, producing a hydrogel with good cohesive properties [27].

Table 1. Formulation of chemically cross-linked hydrogels

Compositions (%w/w)	**Formulation Code**		
	H1	**H2**	**H3**
PVA	10	15	20
PVP K30	10	10	10
Carbomer 980	1	1	1
Tartaric Acid	1.5	1.5	1.5
Deionized water	77.5	72.5	67.5

The polymer solution was made by mixing PVA and PVP K30, then heated at 80^{o}C until a clear solution was obtained. Then, the carbomer (prepared by swelling it overnight on the deionized water) and tartaric acid were added to the solution. Finally, the mixture was poured into the petri dish and dried for two days at 37^{o}C. After drying, the hydrogels were separated from the petri dish and heated at 120^{o}C for two hours [25].

2.3 Fabrication of physically cross-linked hydrogels

Physically cross-linked hydrogels were prepared by a freezing-thaw method according to the procedure explained in the previous study [28]. An aqueous solution containing 1.5% (w/v) SA and 10% (w/v) PVA was dissolved using deionized water. Different

proportions of SA and PVA (**Table 2**) were mixed and vortexed until fully dissolved. A proper amount of the mixture was poured into a petri dish, freezing at -20^{o}C for 18 h and thawing at 25^{o}C for six hours (three continuous cycles).

Table 2. Formulation of physically cross-linked hydrogels

Compositions (%w/w)	**Formulation Code**	
	H4	**H5**
Sodium alginate (1.5%)	30	70
PVA (10%)	70	30

2.4 Swelling studies of hydrogels

The swelling of hydrogel was investigated using a 1 cm^2 hydrogel film over 24 hours at room temperature. The hydrogel was prepared using the methods previously explained in Sections 2.2 and 2.3. Each dry hydrogel was weighed (m_o) and placed in a glass containing 30 mL of PBS (pH 7.4). At intervals 0.5, 1, 2, 3, 4, 5, 10, 15, 30, 45, 60, 120, 180, 240, 300, 360, 420, 480, and 1440 minutes, each hydrogel was removed from the excess solution of PBS using tissue paper and then weighed (m_t). The percentage of swelling was calculated by using the following equation:

$$\% \text{ Swelling} = \left(\frac{mt-mo}{mo}\right) \text{ x } 100\% \qquad (1)$$

2.5 Determination of gel fraction

The obtained hydrogel was dried in an oven at 50^{o}C for 24 h and weighed as W_0. Then, soaked the hydrogel in distilled water for 24 h and dried again at 50^{o}C and weighed as W_e [28]. The gel fraction (GF%) was calculated by using the equation below:

$$\text{Gel fraction (GF\%)} = (We/Wo) \text{ x } 100 \qquad (2)$$

2.6 Water vapor transmission

Water vapor transmission (WVT) studies were carried out using glass vials as the transmission cells. About 1 g of anhydrous fused calcium chloride was initially put into dried glass vials. Then, the hydrogel was taped over the brim of the transmission cells. Furthermore, the vials were weighed and then located to the desiccators containing saturated potassium chloride solution [29]. After the predetermined time, the transmission cells were weighed again, and the WVT was determined using the following equation:

$$\text{WVT} = \frac{\text{mass of the vial x thickness of the hydrogel}}{\text{surface area of the hydrogel}} \qquad (3)$$

2.7 Surface pH

The surface pH test was carried out by weighing 20 mg of hydrogel into a beaker containing 50 mL of distilled water. Then, the hydrogel was let rise at room temperature for 15 minutes, and the combined electrode near the surface of the HFMs was measured after an equilibration time of 1 minute [6].

2.8 Moisture absorption ability

Moisture absorption ability was conducted by using a 1 cm^2 hydrogel. The hydrogels were then placed in three different conditions of relative humidity (RH), i.e., desiccators containing magnesium chloride (33% RH), sodium nitrite (69% RH), and potassium sulfate (97% RH). Every 24 hours within 14 days, each hydrogel was weighed, and the percentage of moisture absorption ability was calculated by using the equation below:

$$\%\text{MAA} = \frac{\text{the final mass of hydrogel} - \text{the initial mass of hydrogel}}{\text{the initial mass of hydrogel}} \qquad (4)$$

2.9 Fabrication of HFMs

HFMs were fabricated using the same method explained in Sections 2.2 and 2.3, except for using a petri dish as the mold. Each 0.5 g of every formula, as shown in **Table 1** and **Table 2**, was poured onto the mold (Silicone template ST-08 10x10 array, 700 µm height) and degassed using a centrifuge at 3500 rpm for 10-20 minutes. For the chemical cross-linking process, each silicone mold containing the formula (**Table 1**) was dried for two days at 37ºC. After drying, the HFMs were separated from the silicone mold and heated at 120ºC for two hours. Meanwhile, for the physical cross-linking process, every mold containing formula (**Table 2**) was frozen at -20ºC for 18 h and thawed at 25ºC for six hours, repeatedly for three continuous cycles.

2.10 Characterisation of HFMs

The penetration test was carried out using eight layers of Parafilm® (≈1 mm; 126 µm/layer). HFMs were attached and lowered onto the eight sheets of Parafilm® and given a load until it met the required force of 32 N, exerted and held for 30 seconds. Following the penetration test, the Parafilm® sheets were unfolded. Then, the number of holes formed in each layer of Parafilm® was counted [24][30].

A mechanical strength test was carried out using a similar method to the penetration test. The HFMs was placed on eight layers of Parafilm® and given a load of 32 N for 30 seconds. The microneedle height before and after the mechanical strength test was compared by using the optical microscope (Olympus® CX23 LED) and measured using Image J (National Instrument of Health, USA) [31].

2.11 Fourier Transform Infrared (FTIR) spectroscopy analysis

FTIR spectroscopy was used to investigate the compatibility of ABZ with all types of

excipients used in this study. All the samples were analyzed using FTIR Accutrac FT/IR-4100 Series (Jasco, Essex, UK) [6].

2.12 Saturation Solubility

The saturation solubility of ABZ was carried out using the shake-flask method [4]. An excess drug was added to the solvent in glass vials. Then, all vials were vortex-mixed for 10 min and ABZ was added to the vial continuously until the solvent was saturated. The vial was then placed in a rotary incubator at 37^{o}C for 24 hours at 40 rpm. After 24 hours, each sample was analyzed using ultraviolet-visible (UV-Vis) spectrophotometry. Solvents chosen and tested in this study included several organic solvents, i.e., PEG 400, PEG 600, ethanol, glycerol, and propylene glycol. This test was also carried out using water to compare the solubility of ABZ in water and other organic solvents.

2.13 Swelling studies of HFMs in organic solvents

The swelling of HFMs in potential organic solvents was conducted using the same method previously explained in Section 2.4. HFMs was weighed (m_o), then placed in a glass containing 30 mL of chosen organic solvents. At regular intervals, each HFM was removed from excess solvents using tissue paper and then weighed (m_t). The swelling percentage was calculated by using equation (1).

2.14 Formulation of the liquid reservoir

The liquid reservoirs were prepared according to the formulation shown in **Table 3**. In various concentrations, PEG 400 and PEG 600 were poured into the glass vial. Then, ABZ was added to the mixture and vortex-mixing vigorously until homogenous.

Table 3. Formulation of ABZ liquid reservoirs

Compositions (%w/w)	Formulation Code		
	F1	F2	F3
ABZ	1	1	1
PEG 400	99	-	49.5
PEG 600	-	99	49.5

2.15 Ex vivo permeation study

All the animals used in this study were treated based on the protocol approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Hasanuddin University, Indonesia. One day before the *ex vivo* permeation study, the skin hair of a euthanized rat was shaved. The abdominal skin was cut out, and the fat layer was removed from the skin. Then, the skin was washed using PBS solution (pH 7.4) repeatedly, and the skin thickness was measured using a digital caliper (Taffware® LCD-XY, Indonesia). Afterward, the skin was wrapped in aluminium foil and placed in the freezer (-20°C) for further testing.

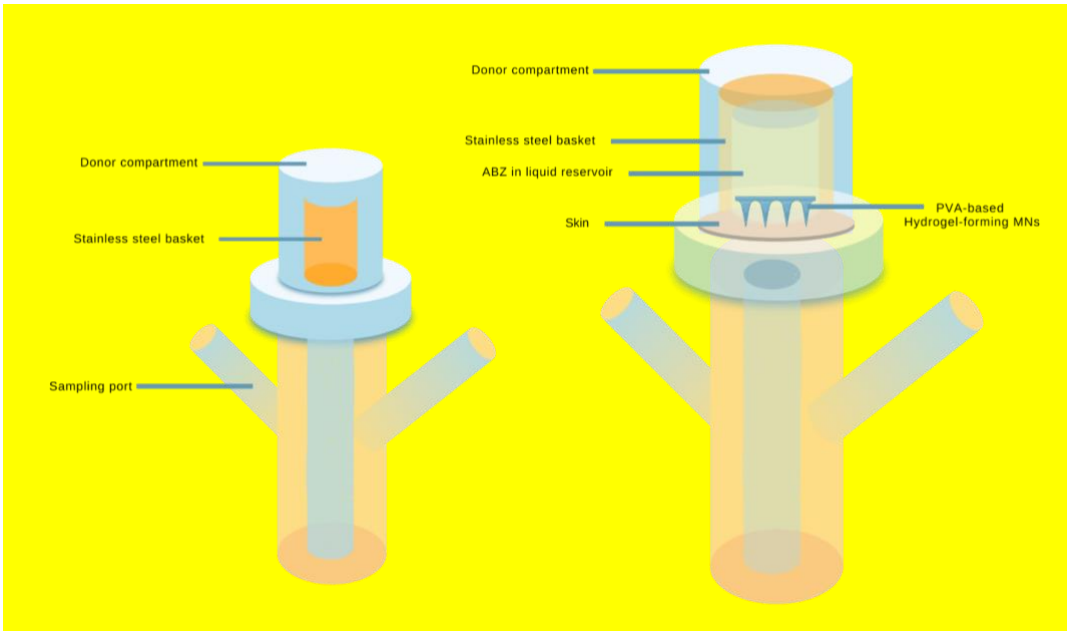

Figure 1. Schematic representation of modified Franz cell setup for *ex vivo* permeation study using stainless steel basket cylinder.

Ex vivo permeation test was carried out using 2% of Tween80® dispersed in PBS (pH 7.4) at 37°C to ensure sink conditions [31]. Using Tween80® as a surfactant will improve the solubility of ABZ during the dissolution studies. In addition, a dissolution medium containing surfactant can better simulate *in vivo* conditions compared to other non-physiological agents or organic solvents [32]. Unfortunately, the conventional Franz cell setup is unsuitable for investigating the permeation ability of liquid reservoirs incorporated with HFMs. A traditional Franz cell setup limits the liquid reservoir's movement to permeate into the skin through the MNs. Then, the various modifications of the Franz cell setup were done using the method described in the previous study [4]. The schematic representation of the modified Franz cell setup was shown in **Fig.1**. In order to calculate the amount of drug release, the samples were then analyzed using UV-Vis spectrophotometry at the maximum wavelength. The cumulative drug release was then fitted into different mathematical kinetic models, as seen in the equations below [33]:

Zero-order kinetics	Q_t	$= Q_0 + K_0t$	
First order kinetics	$\ln Qt$	$= \ln Q_0 - K_1t$	
Higuchi model	Q_t	$= K_H \times t^{1/2}$	
Korsmeyer-peppas model	Q_t	$= Kt^n$	
Hixson-crowell model	$Q_0^{1/3}$	$= Q_0^{1/3} - Q_t^{1/3} = K_st$	(5)

Q_t (%) showed the percentage of drug released at the time (t), Q_0 is the starting value of Q_t and n is the diffusion release exponent. The value of flux (J) and coefficient of permeation (Kp) can be calculated using Eq. (6) [34]. All data of permeation results were calculated using DDsolver.

$$J = Q/A \times t$$

$$Kp = Q/[A \times (C0 - Ci)] \quad (6)$$

2.16 Skin integrity test

A skin integrity test was conducted to investigate the effect of HFMs application after 24 hours of *ex vivo* permeation study [35]. The skin's integrity was evaluated using FTIR spectroscopy at a wavelength of 400 to 4000 cm^1 using FTIR Accutrac FT/IR-4100 Series (Jasco, Essex, UK). At the end of *the ex vivo* permeation study, the skin was washed and analyzed using FTIR spectroscopy. Furthermore, the untreated skin was used as a negative control.

2.17 Statistical analysis

All methods were calculated using Microsoft® Excel® 2019 (Microsoft Corporation, Redmond, USA) to determine the mean and SD. Statistical analysis was performed using IBM® SPSS® Statistics 26.0 (IBM, Armonk, New York, USA). Based on statistical analysis, $p \leq 0.05$ was considered statistically significant in all cases. Premium GraphPad Prism® version 9.2 (GraphPad Software, CA, U.S.A.) was used to create graphs from obtained data.

3. Results and discussions

3.1 Fabrication of hydrogel and in vitro swelling studies

HFMs offer a promising better administration of drugs with the minimally invasive approach that adequately enhances the drug's therapeutic effect due to its capability to form a hydrogel network between each polymeric material [25]. In this study, PVA is essential since it is a water-soluble polymer and slightly soluble in ethanol, implying that it has a high potential as a good hydrogel base material. This polymer constitutes a hydrophilic structure, making it capable of taking up a large amount of water in its

three-dimensional polymeric network. Combined with other polymers, such as PVP K30 and carbomer, the formed hydrogel will potentially provide better swell behavior when inserted into the skin due to the presence of interstitial fluid [36]. Generally, the swelling study is one vital parameter to determine the characteristic of the HFMs. The swelling properties of the hydrogel are defined by several factors, such as the polymer concentration, cross-linker type, or cross-linking ratio. In this case, the concentration of PVA used was variated (**Table 1** and **Table 2**) to determine its effect on hydrogel's swelling properties [37]

The swelling profile of hydrogel in PBS (pH 7.4) was presented in **Fig.2.A**. PBS was chosen as the standard swelling medium to represent the interstitial fluid of the human body [28]. The combined formula of PVA and SA (H4 and H5) was dissolved immediately after contact with the PBS solution (data not shown). The ideal formula of HFMs should maintain its integrity and have good swelling properties in PBS (pH 7.4) [8]. Thus, the physically cross-linked hydrogel formulations were omitted from further studies. Otherwise, the formula containing PVA, PVP, carbomer, water, and tartaric acid (H1, H2, and H3) showed pleasant swelling in PBS. The covalent bonds of those polymers caused the HFMs to not dissolve in the interstitial fluid yet showed high swelling behavior [28].

Fig.2.A compares the swelling percentage (%) of H1, H2, and H3. Based on the results, it is evident that the swelling rate of H1 was superior when compared to the other formulations. Statistical analysis showed that the swelling percentage of H1 was significantly higher than H3 ($p<0.05$). This observation is supported by previous research that explained about the PVA concentration, which was known to substantially affect the swelling behavior of hydrogel. Higher PVA concentration means more sites are available for cross-linking [38]. This can be seen in the lower swelling rate of H2

and H3, shown in **Fig.2.A,** due to the lower PVA concentration than H1. An increase in PVA concentration will lead to the rise of medium viscosity, thereby limiting PVA chain movement. Hence, this will hinder the ability of the polymer matrix to absorb water [39].

3.2 Gel fraction of hydrogel

Generally, the gel fraction (GF) describes the flexibility of the hydrogel. Hydrogel with low flexibility may indicate complex characteristics and difficulty in absorbing liquid, including interstitial fluid [28]. GF also calculates the degree of grafting in each hydrogel formula [40]. Based on the GF determination test, it was found that the GF (%) of H1 (95.45 ± 0.26%), H2 (87.70 ± 0.35%), and H3 (85 ± 0.07%) were in the acceptable range. H1 and H3 differed significantly in GF percentage ($p < 0.05$). The obtained result showed that the GF% monotonically decreased with the increase in PVA concentration.

Furthermore, the GF% provides more information about the cross-linking process' effectiveness in forming an insoluble fraction. As shown in **Table 1**, H1 only consist of 10% of PVA (%w/w) and H3 of PVA (%w/w) up to 20%. The increase in GF percentage implies that this parameter primarily depends on the concentration of PVA, which leads to better cross-linking properties and a higher ability to form an insoluble fraction [41]. A further increase in PVA concentration of about 5% (%w/w) could decrease the swelling capacity due to the viscosity increase, limiting the PVA chain and reducing the water absorption capacity [39].

3.3 Water vapor transmission

WVT test was conducted to determine the hydrogel's permeability characteristics,

showing the quantity of moisture transmitted through the unit area of the hydrogel in unit time. After seven days, the WVT rate turned out to be 0.53 ± 0.04 mg.cm/cm^2 at 24 h, indicating that the H1 formulation was permeable to water vapor, probably due to the PVP content which is one of the hygroscopic polymers. However, this WVT rate is still considered low and potentially indicates the hydrogel's long-term stability [42][29].

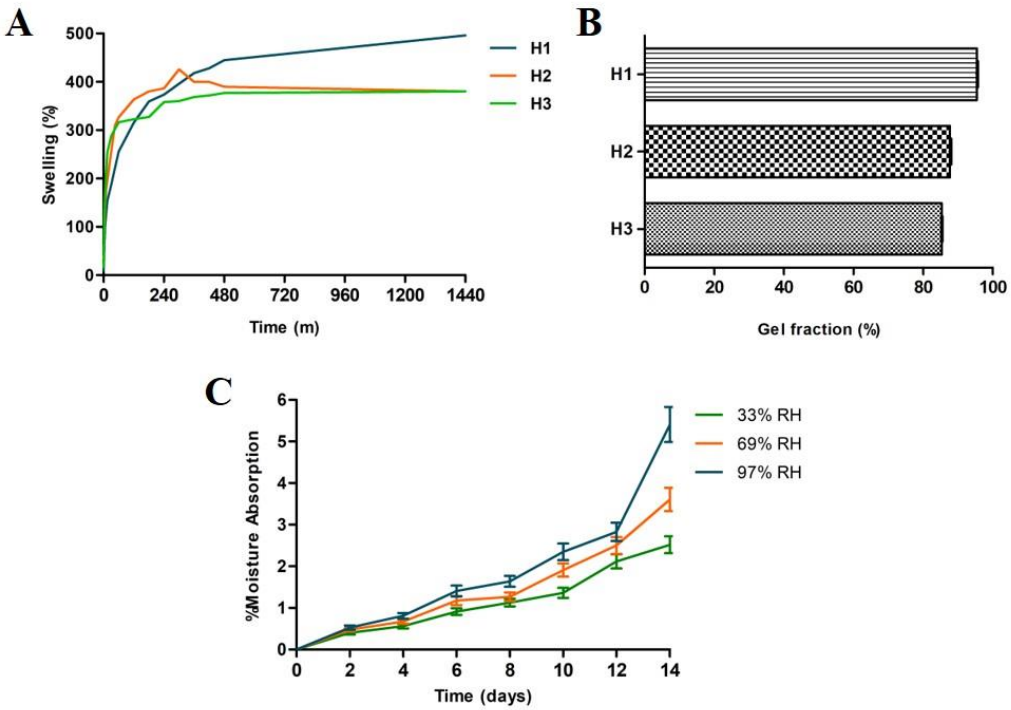

Figure 2. (a) Swelling percentage of HFMs in PBS (pH 7.4); (b) Gel fraction percentage of HFMs (means ± SD); (c) Moisture absorption of H1 at 33% RH, 69% RH, and 97% RH (means ± SD).

3.4 Surface pH

Investigating the surface pH of HFMs is very important to ensure that the surface pH of HFMs, which is directly attached to the skin, could not possibly irritate during application [43]. Inappropriate pH values of preparations can potentially irritate the skin, especially when applied to the skin for an extended time. Thus, it is essential to conduct the surface pH test of HFMs. The result showed that the surface pH value of H1 is about 5.32 ± 0.49, close to the skin's pH around 5.8 [44]. This implied that the HFMs

could be applied to the skin without causing any irritation.

3.5 Moisture absorption ability

Moisture absorption indicated hydrogel's affinity to uptake the external water molecules. In addition, the higher moisture absorption rate may affect the functionality of MNs under storage conditions. Based on the results shown in **Fig.2.C**, the higher RH values directly affect the increase of moisture absorption ability of H1. After 14 days of testing, all formulas had a moisture absorption percentage of <10%. It is probably due to the existence of PVP in a hydrogel formulation. PVP is a unique polymer with a strong moisture absorption ability due to its hygroscopic characteristic. In this case, the PVP used in the formulation will directly affect the moisture absorption ability of the hydrogel [45]. Furthermore, the changes in RH can also affect the ability of the hydrogel to absorb the external water. Generally, the higher RH in the environment will cause a higher value of moisture absorption [46]. However, the moisture absorption of H1 was still considered low compared to other MNs formulas containing PVP that gained up to 14-16% of moisture absorption [42].

3.6 Characterisation of HFMs

Representative H1 of microscopic images are shown in **Fig.3** and **Fig. 5.** The penetration and mechanical test have been carried out to determine the strengths and hardness characteristics of HFMs in correlation with their penetration ability to the skin. The formula of H1 was cast onto the mold, dried at 37^{o}C for two days, and then heated at 120^{o}C for two hours for cross-linking step. The penetration test showed that each HFMs H1 replicated could penetrate up to the fourth layer of Parafilm® (504 µm) with an average percent penetration reaching up to 63% of the microneedle height (**Fig.3.C**). Along with that percentage, it indicates that HFMs H1 can penetrate through the stratum

corneum, passes through the epidermis into the dermis, as the epidermis has a thickness of up to 180 µm [47]. In the case of 32 N applied force, the penetration of the needles into the skin was at its greatest, which showed more than 50% penetration up to the fourth layer of Parafilm®. This result indicates that HFMs H1 was probably hard enough to penetrate and transverse intact to the *stratum corneum*. The obtained result of the mechanical strength test showed a percentage decrease in the height of HFMs H1. After the mechanical strength test, the size of HFMs H1 decreased by only 7.14% of its total height **(Fig.3.D)**.

3.7 Physicochemical characterisation of ABZ and HFMs using FTIR spectroscopy

As shown in **Fig.3.E**, ABZ was characterized using FTIR spectroscopy. The spectra showed the N-H stretching in 3350-3310 cm^{-1} and the peak at 3332,99 cm^{-1}. It was also noticed that the valence vibrations of the aromatic were found in the range of 1650-2000 cm^{-1}. Around 1500-1700 cm^{-1}, the valence vibration of the group carboxyl C=O from the ester group appeared. Moreover, the valence vibrations of the C-O stretching were identified in the 1250-1310 cm^{-1} from group ester, and the peak showed at 1192,01 cm^{-1}.

In the fabrication of hydrogel for drug administration, the cross-linking process can directly affect the properties of each component of HFMs. To determine the cross-linking effects on the structure of each polymer, infrared (IR) spectroscopy was used in this study. The spectra of HFMs containing polymer and cross-linker agent were presented in **Fig.3.E** As for the comparison between pre- and post-crosslinked HFMs, the spectra were shown in **Fig.3.F.** The critical difference between IR spectra of pre- and post-crosslinked HFMs can be found in the presence of new band between 1750

and 1500 cm^{-1}. This range was known as the carbonyl region. The pre-crosslinked HFMs presented only a single peak in the carbonyl region. Thus, the spectra of post-crosslinked HFMs showed two different carbonyl peaks. The new peak was shown at ca. 1730 cm^{-1}, which can be identified as new ester cross-link bonds. This unique bond can be formed due to the esterification process between the reaction of hydroxyl groups of PVA and carboxylic groups of tartaric acid during the cross-linking process [48].

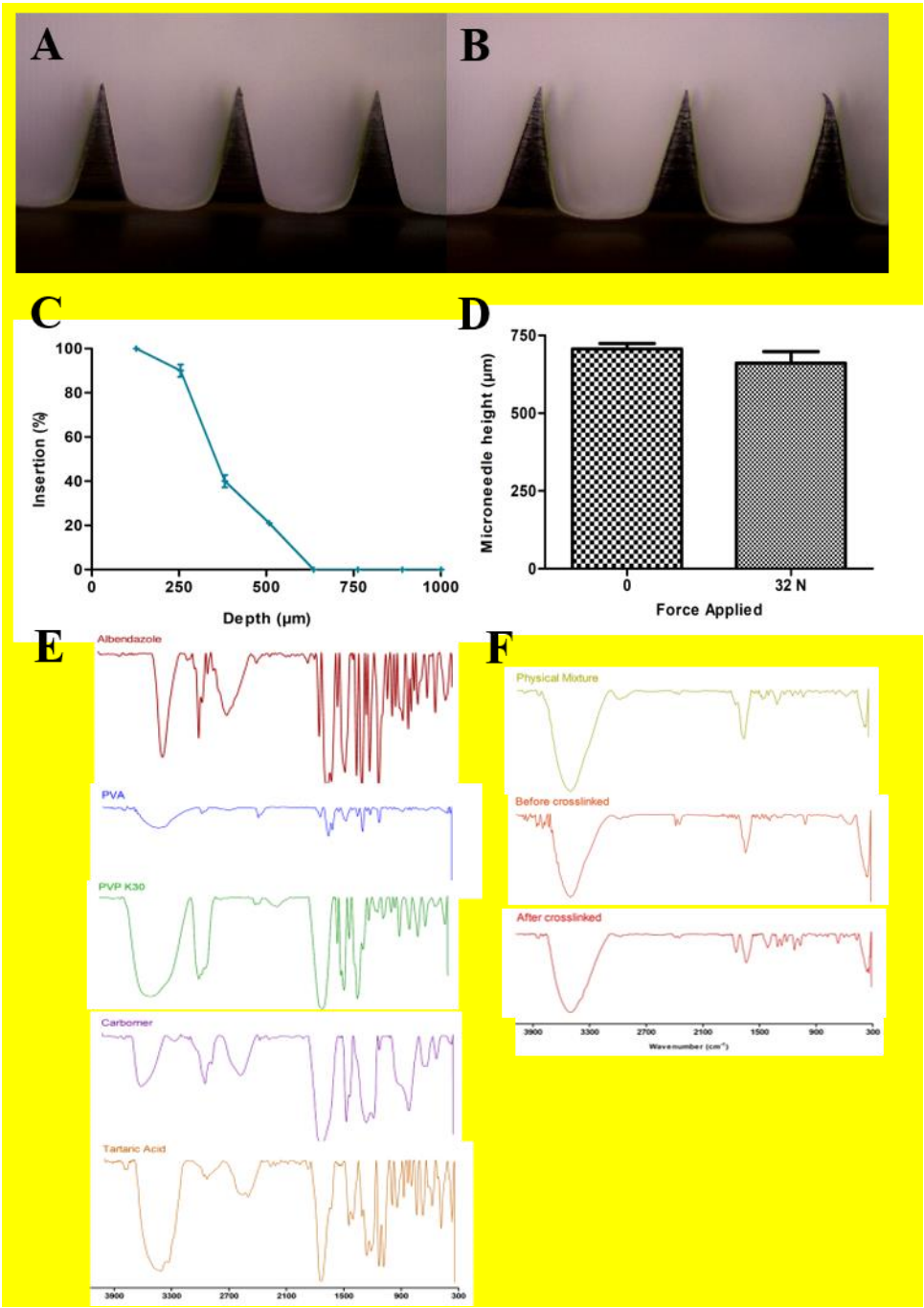

Figure 3. Microscopic observation of HFMs H1 (4x magnification) (a) before mechanical strength test; (b) after mechanical strength test; (c) Penetration test results of HFMs H1 (means + S.D., n = 3); (d) Comparison of HFMs H1 height before and after mechanical strength test (means ± S.D., n = 3); FTIR spectra of (e) ABZ's spectra and each composition of H1; (f) physical mixture of HFMs H1, before and after-crosslinked process of H1.

As shown in **Fig.3.F**, the intermolecular interactions of tartaric acid molecules with PVA polymer chains are shown by several inter- and intramolecular cross-links formed. The position of the band shifted, and the absorbance of this band increased significantly with the increase of tartaric acid content, indicating an increase in cross-link density in the structure. The absorbance of the carbonyl group increased with increasing TA concentration due to the esterification process. The cross-linking of the PVA chains reduces the hydroxyl groups of the PVA chains and forms new cross-linked ester bonds with the carbonyl strain [48].

3.8 Saturation solubility

The solubility of ABZ in some potential organic solvents was carried out to determine the most suitable reservoir medium to use incorporated with HFMs H1. The saturation solubility of ABZ was conducted to provide a more detailed understanding of the solubility of ABZ. The solubility was tested in several organic solvents that could potentially enhance the absorption and bioavailability of ABZ in the liquid reservoir.

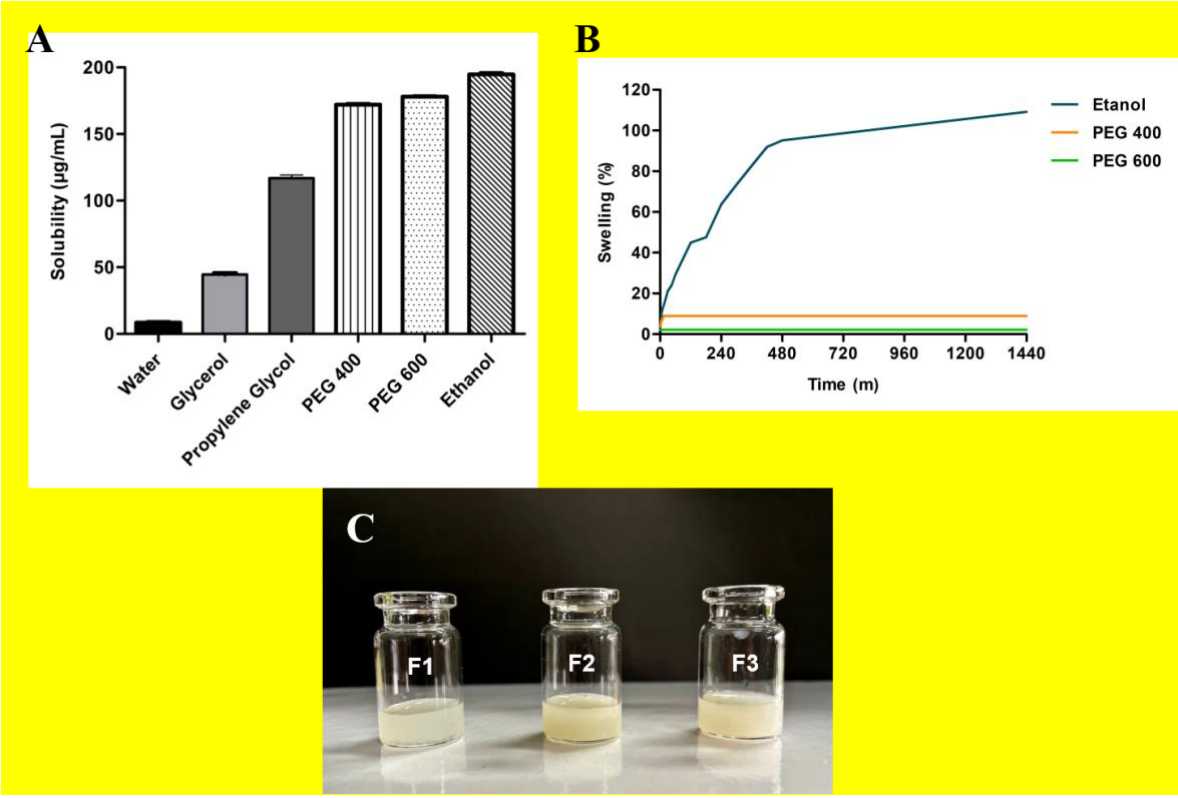

Figure 4. (a) Solubility of ABZ in several organic solvents; (b) Swelling percentage of HFMs H1 in potential organic solvents; (c) Physical appearance of the liquid reservoir.

As shown in **Fig.4.A**, the results showed that the highest ABZ solubility was found in a mixture of ABZ with ethanol, PEG 400, and PEG 600. The results of ABZ solubility in ethanol compared to PEG 400 and PEG 600 were significantly different ($p < 0$ 0.05). The ABZ solubility in ethanol was shown to be the highest. In contrast, the solubility of ABZ at PEG 400 and PEG 600 was not statistically significant ($p > 0.05$). Therefore, to determine the most appropriate solvent for the liquid reservoir of ABZ, those three different solvents were used for the swelling test using HFMs H1.

3.9 Swelling studies of HFMs in organic solvent and preparation of liquid reservoir

Drugs with poor solubility in water face major issues during pharmaceutical fabrication. Those drugs commonly need to be dissolved into the gastrointestinal fluids to enhance

oral bioavailability. The poorly soluble drug often results in limited solubility in gastrointestinal fluid and lower oral bioavailability and absorption [49]. Many strategies have been developed to improve the bioavailability of poorly soluble drugs, including loading the drug in a liquid reservoir by using a non-polar solvent. In the liquid reservoir, adding a non-polar solvent will reduce the system's polarity, enhancing the solubility of the poorly soluble drug [4].

In order to fabricate the liquid reservoir, a swelling test of HFMs H1 in 100% organic solvent was carried out in PEG 400, PEG 600, and ethanol to identify the suitable reservoir medium for the liquid reservoir. The results showed that the highest percentage of swelling rate was found in ethanol compared to PEG 400 and PEG 600, and the obtained results were statistically significant ($p < 0.05$). A suitable reservoir medium in a liquid reservoir incorporated with HFMs must unfound the swelling of HFMs, to ensure that HFMs can only swell upon contact with the body's interstitial fluid [4]. Therefore, based on the results of the swelling test in 100% organic solvents, as shown in **Fig.4.B**., PEG 400 and PEG 600 were chosen as the most suitable reservoir medium.

3.10 Ex vivo permeation study

The *ex vivo* permeation test was conducted to determine the permeation profile of each formula of liquid reservoir integrated with HFMs H1. The accumulative permeation of ABZ at 24 hours from each formula was F1 (865.43 ± 5.95 µg/cm^2), F2 (869.06 ± 25.46 µg/cm^2), and F3 (971.23 ± 11.77 µg/cm^2) (**Fig.5.A**).

Table 4. Permeation parameter of ABZ liquid reservoir in infinite dose condition

Formula	**Parameter**

	P (cm/h) ± SD	J (µg/cm2/h) ± SD
F1	285.39 ± 4.13	4.40 ± 0.06
F2	290.26 ± 0.00	4.47 ± 0.00
F3	303.57 ± 13.32	4.68 ± 0.21

The value of the permeation coefficient (*P*) in **Table 4** also describes the amount of ABZ permeated per unit of concentration described in cm/hour. As evident from the results in **Fig.5.A**, F3 provided a better drug flux across the skin than F1 and F2. Therefore, considering the cumulative amount of ABZ permeated through the skin membrane, the combination of HFMs H1 and reservoir F3 was consider as the most appropriate combination for delivering ABZ through the transdermal route by using HFMs and liquid reservoir in this study.

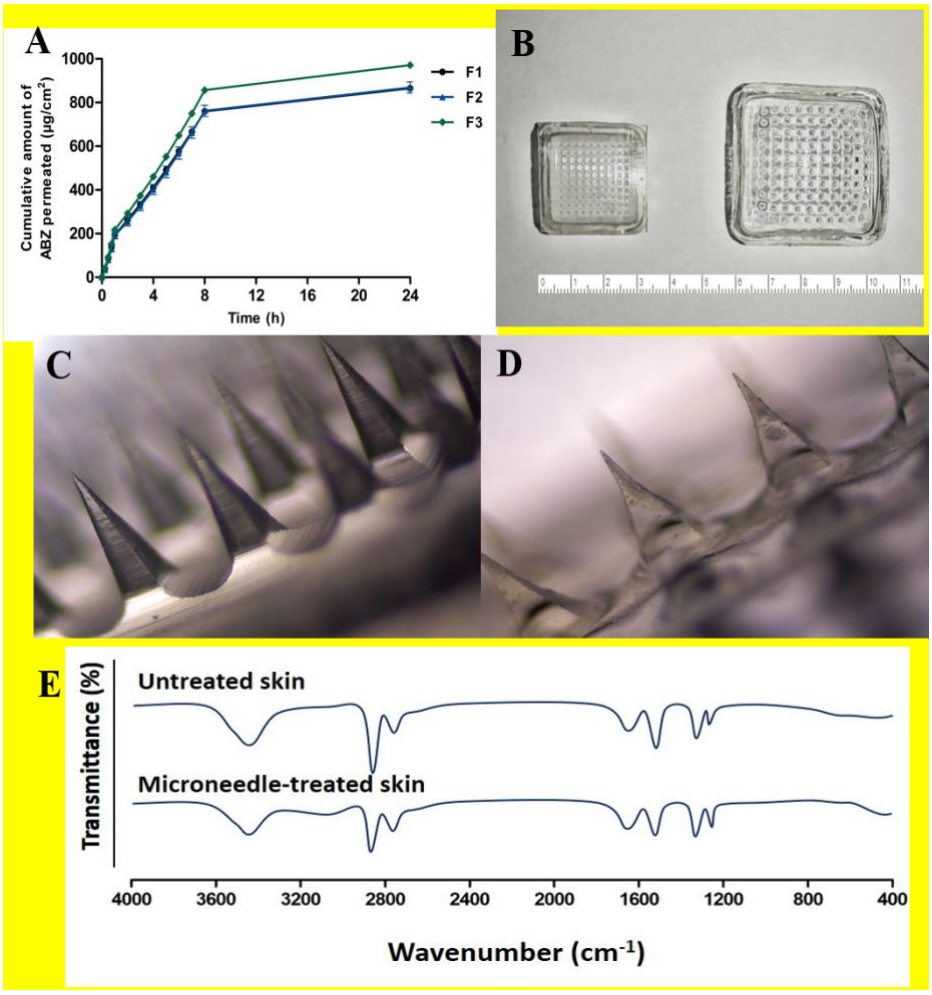

Figure 5. (a) *Ex vivo* permeation profiles of HFMs H1 incorporated with the liquid reservoir F3; (b) Macroscopic comparison of HFMs pre- and post-swelling; Microscopic view of HFMs H1 (4x magnification); (c) before *ex vivo* permeation test; (d) after *ex vivo* permeation test. (e) FTIR spectra of integrity evaluation (comparing the untreated and microneedle-treated skin).

To determine the mechanism of ABZ release from HFMs incorporated with a liquid reservoir, the permeation data were analyzed using several different kinetic models (zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas). The results showed the release of ABZ following the Korsmeyer-Peppas kinetic model with a correlation coefficient value (R2) is 0.95. In the Korsmeyer-Peppas kinetic model, the value of n determines the mechanism of drug release. The value of n found in this permeation test is 0.217. The value of $n < 0.5$ indicates the mechanism of drug release by Fickian diffusion [50][51][52]. In that sense, the release of ABZ depended on the diffusion of liquid that occurs through the swollen matrix of HFMs, which could be generated by the relaxation of polymer chains [53].

3.11 Skin integrity test

The integrity evaluation result using FTIR is shown in **Fig. 5.E**. Comparing the untreated and microneedle-treated skin, a significant difference was found in the presence of new peaks at 2915 cm^{-1} and 2760 cm^{-1} in the untreated skin. These ranges were also known as hydrocarbon areas, which were present due to the asymmetric hydrocarbon stretching and symmetric CH_2 stretching. This hydrocarbon area represents the ceramide and the fatty acid in the stratum corneum. Furthermore, the amide I and amide II bonds were also discovered in the corneocytes' keratin at 1679 cm^{-1} and 1513 cm^{-1}. According to **Fig.5.E**, all those peaks were also present in microneedle-treated skin with unsignificant changes in all intensities. Based on the obtained result above, it

was concluded that the administration of HFMs did not change the integrity of the skin after 24 hours of application and is considered safe to be used in drug administration.

4. Conclusion

HFMs incorporated with a liquid reservoir containing ABZ have been successfully formulated using the combination of PVA, PVP K30, carbomer, and tartaric acid as chemical cross-linker. The obtained result of HFMs has been evaluated based on the mechanical properties, swelling profile, gel fraction, water vapor transmission, surface pH, moisture absorption ability, FTIR studies, *ex vivo* permeation study, and skin integrity test. The overall result of this study showed the promising advantages of using HFMs incorporated with a liquid reservoir to overcome the limitations of ABZ in oral administration. Furthermore, *in vivo* pharmacokinetic, pharmacodynamic, and toxicity tests should be conducted in appropriate animal models to investigate this innovation's effectiveness and safety for future commercial use.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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226247195 (Journal of Biomaterials Science: Polymer Edition) Your submission has been accepted

1 message

Stuart Cooper <em@editorialmanager.com> Fri, Dec 9, 2022 at 6:18 AM
Reply-To: Stuart Cooper <coopers@chbmeng.ohio-state.edu>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Ref: JBS-D-22-00407R1
226247195
Development of Hydrogel-Forming Microneedles for Transdermal Delivery of Albendazole from Liquid Reservoir
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 Dec 08, 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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