BUKTI SUBMIT MANUSKRIP

Universitas Hasanuddin

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

Langmuir - Manuscript ID la-2022-02754m

1 message

Langmuir <onbehalfof@manuscriptcentral.com> Mon, Oct 10, 2022 at 6:47 PM
Reply-To: admin-journals@services.acs.org
To: nuuraanisah@untad.ac.id, Sulistiawati@student.farmasi.unhas.ac.id, andi.dian.permana@farmasi.unhas.ac.id

10-Oct-2022

Journal: Langmuir
Manuscript ID: la-2022-02754m
Title: "Development of solid lipid nanoparticles loaded polymeric hydrogels containing antioxidant and photoprotective bioactive compounds of Safflower (Carthamus tinctorius L.) for improved skin delivery"
Authors: Aanisah, Nuur; Sulistiawati, Sulistiawati; Djabir, Yulia Yusrini; Asri, Rangga Meidianto; Sumarheni, Sumarheni; Permana, Andi Dian
Manuscript Status: Submitted

Dear Dr. Permana:

Your manuscript has been successfully submitted to Langmuir.

Please reference the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your contact information, please log in to ACS Paragon Plus with your ACS ID at http://acsparagonplus.acs.org/ and select "Edit Your Profile" to update that information.

It is our policy that all authors must approve submission of this manuscript. If you are a co-author, we hope that you approve of the manuscript and its submission to Langmuir. If you do not approve of this submission, please notify the Editor-in-Chief office, eic@langmuir.acs.org , immediately with an explanation. If you do approve this manuscript submission, no further action is necessary.

You can view the status of your manuscript by checking your "Authoring Activity" tab on ACS Paragon Plus after logging in to http://acsparagonplus.acs.org/.

Journal Publishing Agreement and Copyright

Upon acceptance, ACS Publications will require the corresponding author to sign and submit a Journal Publishing Agreement. This agreement gives authors a number of rights regarding the use of their manuscripts. At acceptance, the corresponding author will receive an email linking through to the Journal Publishing Agreement Wizard, which helps you select the most appropriate license for your manuscript.

For more information please see:
https://pubs.acs.org/page/copyright/journals/jpa_faqs.html

ACS Authoring Services

Did you know that ACS provides authoring services to help scientists prepare their manuscripts and communicate their research more effectively? Trained chemists with field-specific expertise are available to edit your manuscript for grammar, spelling, and other language errors, and our figure services can help you increase the visual impact of your research.

Visit https://authoringservices.acs.org to see how we can help you! Please note that the use of these services does not guarantee that your manuscript will be accepted for publication.

Thank you for submitting your manuscript to Langmuir.

Sincerely,

Langmuir

BUKTI REVIEW DARI REVIEWERS

Universitas Hasanuddin

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

Revision Requested for Manuscript ID la-2022-02754m

3 messages

Langmuir <onbehalfof@manuscriptcentral.com> Tue, Nov 29, 2022 at 3:39 AM
Reply-To: heinz-office@langmuir.acs.org
To: andi.dian.permana@farmasi.unhas.ac.id

28-Nov-2022

Journal: Langmuir
Manuscript ID: la-2022-02754m
Title: "Development of solid lipid nanoparticles loaded polymeric hydrogels containing antioxidant and photoprotective bioactive compounds of Safflower (Carthamus tinctorius L.) for improved skin delivery"
Author(s): Aanisah, Nuur; Sulistiawati, Sulistiawati; Djabir, Yulia Yusrini; Asri, Rangga Meidianto; Sumarheni, Sumarheni; Permana, Andi Dian

Dear Dr. Permana:

The reviews for your manuscript are enclosed with this letter. Please consider the reviewers' comments. They have raised points that require significant consideration and revision of the manuscript before it is suitable for publication. However, with adequate response and revision, the manuscript may be acceptable for publication in Langmuir. We would like to receive your revision as soon as possible, by 19-Dec-2022 at the latest. Please use the link below to access a manuscript checklist for guidance in preparing your revision.

http://pubs.acs.org/paragonplus/submission/langd5/langd5_checklist.pdf

Please note that you may receive a follow-up message within 24 hours describing the non-scientific changes you should also make to your manuscript, in accordance with our author guidelines, before you submit the revision. It is important to address these changes in your revision so that processing your manuscript can proceed without further delay.

To revise your manuscript, log into ACS Paragon Plus with your ACS ID at http://acsparagonplus.acs.org/ and select "My Authoring Activity". There you will find your manuscript title listed under "Revisions Requested by Editorial Office." Your original files are available to you when you upload your revised manuscript. If you are replacing files, please remove the old version of the file from the manuscript before uploading the new file.

When submitting your revised manuscript through ACS Paragon Plus, you will be able to respond to the comments made by the reviewer(s) in the text box provided or by attaching a file containing your detailed responses to all of the points raised by the reviewers.

In addition to uploading your revised manuscript file, please upload an annotated copy of the manuscript file that tracks deletions and additions for the benefit of the reviewers and editor. This marked-up manuscript should be uploaded electronically in the File Upload section as "Supporting Information for Review Only". (Note that if a revised Supporting Information file is uploaded, also upload an annotated version of it, again designated as Supporting Information for Review Only.)

Prior to submitting the manuscript, ensure that the manuscript addresses the following points:

Format: Your revised manuscript must adhere to ACS format, especially references. For your convenience you can access the Langmuir guidelines for authors here: http://pubs.acs.org/page/langd5/submission/index.html. Langmuir is now publishing titles in the references. Your revision should include the manuscript titles in your references. Note that in ACS Paragon Plus, authors have the option of embedding graphics into the manuscript or supplying graphics separately.

Table of Contents Graphic: Langmuir requires a Table of Contents Graphic. The graphic requested for the table of contents entry could be in the form of a structure, graph, drawing, SEM/TEM photograph, or reaction scheme. Instructions are available at http://pubs.acs.org/page/langd5/submission/index.html.

Supporting Information: If the manuscript is accompanied by any supporting information for publication, a brief description of the supplementary material is required in the manuscript. The appropriate format is: Supporting Information. Brief statement in nonsentence format listing the contents of the material supplied as Supporting Information.

Funding Sources: Authors are required to report ALL funding sources and grant/award numbers relevant to this manuscript. Enter all sources of funding for ALL authors relevant to this manuscript in BOTH the Open Funder Registry tool in ACS Paragon Plus and in the manuscript to meet this requirement. See http://pubs.acs.org/page/4authors/funder_options.html for complete instructions.

ORCID: Authors submitting manuscript revisions are required to provide their own validated ORCID iDs before completing the submission, if an ORCID iD is not already associated with their ACS Paragon Plus user profiles. This iD may be provided during original manuscript submission or when submitting the manuscript revision. You can provide only your own ORCID iD, a unique researcher identifier. If your ORCID iD is not already validated and associated with your ACS Paragon Plus user profile, you may do so by following the ORCID-related links in the Email/Name section of your ACS Paragon Plus account. All authors are encouraged to register for and associate their own ORCID iDs with their ACS Paragon Plus profiles. The ORCID iD will be displayed in the published article for any author on a manuscript who has a validated ORCID iD associated with ACS Paragon Plus when the manuscript is accepted. Learn more at http://www.orcid.org.

ACS Publications uses Crossref Similarity Check Powered by iThenticate to detect instances of similarity in submitted manuscripts. In publishing only original research, ACS is committed to deterring plagiarism, including self-plagiarism. Your manuscript may be screened for similarity to published material.

Sincerely,

Prof. Hendrik Heinz
Senior Editor
Langmuir
University of Colorado at Boulder
Email:heinz-office@langmuir.acs.org

Reviewer(s)' Comments to Author:

PLEASE NOTE: When reviewers point to specific line numbers in their comments, they are often referring to the line numbers inserted by ACS Paragon Plus in the PDF Proof of the manuscript. The PDF Proof is attached.

Reviewer: 1

Recommendation: Major revisions needed as noted.

Comments:
In this manuscript, quercetin and luteolin, the active components of safflower, were extracted and then encapsulated in a hydrogel based on solid lipid nanoparticles to enhance solubility and skin permeability. It has been proved that this formulation has good antioxidant activity and sunscreen effect. However, there are major problems in the writing of the manuscript, which makes it difficult for readers to read. At the same time, it lacks professional expression and makes mistakes on many common-sense issues. In addition, the design of the material system lacks highlights, and the experimental scheme needs to be improved. The problems in the manuscript are as follows:

1. The abbreviation SPE appears for the first time in the abstract, but its full name is not stated. Meanwhile, the keyword photoprotective does not appear in the abstract.
2. In the manuscript, line 158, FeCl2 is miswritten and line 171, the reversed-phase column size (150 × 4.6 mm, 5 m) should be changed to (150 × 4.6 mm, 5 μm). In line 225, the heating rate of 10 C/min should be changed to 10℃/min. In line 329, the gravity acceleration (980 m/s2) should be changed into 9.8 m/s2. In line 517, SLNs is incorrectly expressed. In table4, the number 0,97 should be changed into 0.97.
3. In the manuscript, italics should be used in vivo and in vitro. In addition, the use of many units in the manuscript should also be standardized, such as μL and mL.
4. There are many figures in the manuscript that are not clear enough, as shown in Figure 6, and it is recommended to redraw. In addition, some column charts have uneven color filling, as shown in Figure 1B, and it is recommended to redraw.
5. The SEM images (Figure 4D) of SLNs formulations lack scale, which is very important. In addition, the author developed a hydrogel formulation based on solid lipid nanoparticles (SLNS), but did not conduct any characterization of the hydrogel morphology, which is not rigorous.
6. In the in vitro penetration experiment of gel (as shown in FIG. 6 and FIG. 8), the time interval of measurement points was set unreasonable. Follow-up measurements should be taken during periods of rapid increase in data.
7. What is the maximum diameter of nanoparticles that can be penetrated through the skin and whether relevant experiments have been done to ensure that the formulation can meet the conditions of skin penetration?
8. The study from the manuscript found that the drug-loaded solid lipid nanoparticle hydrogels showed advantages in many aspects, but achieved almost the same effect in the sunscreen test as the hydrogels directly loaded with drugs. Is it contradictory? What is the significance of this designed drug delivery gel formulation based on solid lipid nanoparticles?

9. Safety is a necessary issue to be considered for cosmetics. Whether the formulation designed by the author will cause allergies, and whether the product will interfere with hormones and reproductive system after penetrating into the skin and entering the human body are all questions to be considered. However, there is no description of these issues in the manuscript.

Additional Questions:
Significance: Moderate (not suitable for Langmuir)

Originality: High (suitable for Langmuir)

Broad Interest: High (suitable for Langmuir)

Technical Quality: Moderate (not suitable for Langmuir)

Is the manuscript appropriate for Langmuir?:

Are the conclusions adequately supported by the data?:

Are the references appropriate and correct?:

Is the English adequate?:

Is the quality of the figures sufficient?:

Are the data statistically significant with appropriate controls included?:

Reviewer: 2

Recommendation: Other

Comments:
Manuscript number: la-2022-02754m

Title: “Development of solid lipid nanoparticles loaded polymeric hydrogels containing antioxidant and photoprotective bioactive compounds of Safflower (Carthamus tinctorius L.) for improved skin delivery”

Authors: Aanisah et al.

The manuscript describes the physicochemical characterization and biological activity studies on some solid lipid nanoparticles. The works are trustworthy and appears to be systematically carried out. However, the manuscript suffers from several lacunae. It needs thorough revision before a final decision be made. The major concerns are the length, number of figures/images and proper interpretation of the experimental results.

Some specific comments:

1. There are several wrongly constructed sentences. Sometimes the statements are self-contradictory.

2. Line 30: In the abstract what do the authors mean by the term “drug”?

3. Line 29-32: It is mentioned that the drug is trapped in the lipid matrix. If it is so, then how could the drug release be faster? And in any drug delivery system, the prerequisite is the process of sustained release.

4. In the abstract the acronyms like “SPE” and “SPF” were used. It is preferred that their full forms would be used in their first appearances. In fact, throughout the entire manuscript, many acronyms were used without their introduction.

5. In the abstract section itself I would expect some comments on the biocompatibility of the formulations. Are they cytotoxic? In fact, authors have carried out the same, however, they remain silent about the biocompatibility, which is considered as one of the important markers of any drug delivery system.

6. The introduction section seems to be quite lengthy and unorganized. It should be more focused and specific. How the present set of works are going to shed new lights over the existing lacunae? What would be the new insights? Authors may mention them at the end of the introduction section.

7. In the introduction section a brief description on “Geleol” would be more impressive for general readers.

8. In the beginning of the experimental section, I would expect the details on the chemicals used, (purity, make and country of origin, etc.) and the instruments.

9. What is the rationale behind preparing sixteen different formulations (Table 2)?

10. Usually, active ingredients from plant products are extracted from the dried and pulverized plant components. What was the rationale behind extracting from the raw plants? Also, I would expect a line of statement as why different proportions of water-alcohol mixtures were used in the extraction procedure.

11. Equation 1: While calculating the yield of extraction, should one take into account the whole flower? In the denominator, should it be Wsafflower or Wsafflower petals?

12. The experimental part is also quite lengthy. It needs to be substantially shortened.

13. Table 2: Which Tween was used as stabilizer? What is the molecular weight of PVA?

14. 2.2.3.1. Particle Size, PDI, and Zeta Potential studies: What was the scattering angle? What is the model number of DLS? What type of laser was used?

15. 2.2.3.4. DSC studies: The scan rate seems to be quite high. Please cite proper references where such high scan rates were used.

16. 2.2.3.6. Scanning Electron Microscope: Please provide some details on the sample preparation. What was the coating agent? What was the applied voltage for SEM analyses?

17. 2.2.3.7. Solubility analysis. The saturated solubility analysis of QU and LU: What are AU and LU?

18. Authors should clearly mention the ethical guidelines regarding the biological studies involving animals.

19. Please mention the positive and negative controls while performing the biological activity tests (biocompatibility and skin permeation tests).

20. Sections 3.1-3.4: These sections are not so relevant, and can be moved to the supplementary section.

21. It is not unexpected that “The maximum DPPH radical scavenging activity was produced by the extract made with 100% ethanol (Figure 1E)”, and in fact this was also my concern regarding the extraction of SPE using different blends of ethanol +water.

22. Figure 3A: Authors should also mention that higher the amount of Tween, smaller were the sizes for the SLNs. Besides, presence of larger quantities of Tweens lead to lower values of PDI.

23. Authors should comment on the stability of the SLN formulations. Have they tried to monitor the size as function of time? Shelf-life is an important marker of drug delivery systems.

24. 3.6.2 FTIR Study and 3.6.4 PXRD Study: I would expect the description on the FTIR/XRD studies of the excipients (precursors) and physical mixtures and subsequent comparison with the different formulations. Location of the drugs in the SLN matrix could also be explained by suitably analyzing the FTIR data.

25. 3.6.3 DSC analysis: interpretation of the DSC data are wrong. Authors should note that the SLNs show characteristic transition peaks in the range of 30-80 deg C. And in fact, it is clear from Figure 4B that there are some transitions taking place. Authors should narrow down their studies within this temperature range.

26. Scale bars/magnification in the SEM images are missing. Authors should also try to compare the DLS and SEM data.

27. Line 489: Particles were not “spherical vesicles”. Vesicles can not be visualized by SEM. I understand that the authors are not dealing with the vesicles.

28. Table 4: I would expect the presentation of regression coefficient values. This would eventually enable readers to understand the superiority of a particular model.

29. Figure 6: In the Y axes of each graph, should it be “%Cumulative release”?

Additional Questions:
Significance: Moderate (not suitable for Langmuir)

Originality: Lowest (not suitable for Langmuir)

Broad Interest: High (suitable for Langmuir)

Technical Quality: Lowest (not suitable for Langmuir)

Is the manuscript appropriate for Langmuir?: Yes

Are the conclusions adequately supported by the data?: No

Are the references appropriate and correct?: Yes

Is the English adequate?: No

Is the quality of the figures sufficient?: No

Are the data statistically significant with appropriate controls included?: Yes

Reviewer: 3

Recommendation: Major revisions needed as noted.

Comments:
Overall, the paper has merit. The work is detailed, thorough and sound research. As the article is written, it is unclear as to what the novelty of the work is.

In addition, there are a number of items with the paper that should be addressed before publication:

1) Line 51: shouldn't the word "cancer" come after the words "third most common"
2) Line 80-82: the authors state that various approaches and carrier systems have been studied, but yet no references of the types of approaches or carrier systems are provided. In addition, the introduction in general does not provide an adequate overview of the different carrier systems developed to date and how their system is an innovation in the technology.
3) Line 265: The 1 h incubation time seems short, why was this short exposure time chosen.
4) Line 336-338: Why was only 290-320 nm wavelengths selected as opposed to the full range of UVA&B wavelengths (280-400 nm)?
5) Line 364-365: The authors state that the data shows "significant increase in TPC and TFC compared to the TPC and TFC achieved with E100, E50, E25 and WE." However, it is unclear where the significance lies, i.e. is it between all ethanol solutions and water or between each ethanol solution and water, etc. Clarification should be provided.
6) Line 368: Figure 1. Any significant differences between the bars should be labeled on the graphs, i.e. which data is considered to be significantly different from other data?
7) Line 405: Figure 2: Significance needs to be identified for the data in graph B. Labeling of some of the other key peaks in the chromatogram (Figure 2A) would be helpful.
8) Line 433-434: The authors state that the higher concentration of Geleol in SLNs increased particle size and PDI of SLNs as evidenced by graphs 3A & B. In general, this does not appear to be true except for the PVA surfactant case. The Tween 80 surfactant seemed to produce a more consistent particle size and PDI regardless of Geleol concentration. Also, no indication on any significant differences between the data presented in Graphs for Figure 3 A-D and graph D is not labeled.
9) Lines 439-443: What is the implication of the fact that their SLNs had zeta potentials in the narrow range of -29.33 and -33.91 mV?
10) Lines 444-447: This statement appears to be correct for the F1-F4 formulations, but not necessarily for the F9-F12 formulations. Please clarify.
11) Lines 504-507: Are the aqueous solubility values significantly different from one another. They appear to be when comparing QU & LU SPEs to SLNs F3 & F4 values, but was a test of significance performed? The n-octanol solubility does not appear to be significant different but a test would need to be performed to verify.
12) Line 508: The authors state "This significant increase was due to..." What significant increase? The aqueous solubility or n-octanol? Since it is unclear whether a test of significance was performed, how can it be stated that it was a significant increase?
13) Lines 526-527: The word "respectively" needs to added immediately after "F3" and "F4". Also, for the in vitro drug release, were the release values significantly different between the SPE and respective SLNs?
14) Figure 6: The y-axis scale are different for graphs A/B compared to C. It may be more helpful to use the same y-axis scale to better illustrate the difference of the drug release amount. It may be better to show 2 graphs - one for QU and one for LU for all 3 carrier platforms, i.e F3, F4 and SPE, as opposed to showing 3 graphs for each carrier platform and QU and LU on the same plot.
15) Line 548: The word "respectively" should be added after LU.
16) Line 554: The word "respectively" should be added after F4.
17) Line 572: The word "respectively" should be added after 4.87%.
18) Line 582: "($p<0.05$)" should be added after the word "significantly".
19) Line 586: The word "respectively" should be added after 1.72%.
20) Figure 8: again the data may be better presented using 2 graphs instead of 3 as mentioned in item 14 (Figure 6) above.
21) Figure 9: Using similar y-axis scale for graphs A-C would be helpful and more clearly illustrate the differences in the data.

22) Line 614: The authors refer to figure 7E for the bioadhesive strength of each hydrogel, yet graph 7E is SPE data. Please clarify. Also, from the SPF data, it looks like the team did not achieve their goal in the research of trying to demonstrate greater protection with the SLNs. This is not properly discussed in the discussion section.

Additional Questions:
Significance: Moderate (not suitable for Langmuir)

Originality: Moderate (not suitable for Langmuir)

Broad Interest: High (suitable for Langmuir)

Technical Quality: High (suitable for Langmuir)

Is the manuscript appropriate for Langmuir?: In Part

Are the conclusions adequately supported by the data?: Yes

Are the references appropriate and correct?: Yes

Is the English adequate?: Yes

Is the quality of the figures sufficient?: No

Are the data statistically significant with appropriate controls included?: Yes

---
FOR ASSISTANCE WITH YOUR MANUSCRIPT SUBMISSION PLEASE CONTACT:
ACS Publications Customer Services & Information (CSI)
Email: support@services.acs.org
Phone: 202-872-4357
Toll Free Phone: 800-227-9919 (USA/Canada only)

Thank you.

PDF Proof.PDF
1227K

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id> Tue, Nov 29, 2022 at 7:57 AM
To: aanisah.nuur@gmail.com

[Quoted text hidden]

PDF Proof.PDF
1227K

Nuur Aanisah <aanisah.nuur@gmail.com> Tue, Dec 6, 2022 at 5:51 AM
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Assalamualaikum kak
Kak, ini utk revisinya dan rebuttal letternya
yang warna merah, belum ada perbaikannya kak 🙏 🙏 🙏

makasihhh kak Dian
[Quoted text hidden]
--
Nuur Aanisah

---

2 attachments

Manuscript-Carthamus-SLNs.docx
1557K

Langmuir Rebuttal Letter.docx
51K

BUKTI SUBMIT HASIL REVIEW

Development of solid lipid nanoparticles loaded polymeric hydrogels containing antioxidant and photoprotective bioactive compounds of Safflower (Carthamus tinctorius L.) for improved skin delivery

Journal:	*Langmuir*
Manuscript ID	la-2022-02754m.R1
Manuscript Type:	Article
Date Submitted by the Author:	10-Dec-2022
Complete List of Authors:	Aanisah, Nuur; Tadulako University Sulistiawati, Sulistiawati; Hasanuddin University Faculty of Pharmacy, Pharmacy Djabir, Yulia Yusrini; Hasanuddin University Faculty of Pharmacy, Pharmacy Asri, Rangga; Hasanuddin University Faculty of Pharmacy Sumarheni, Sumarheni; Hasanuddin University Faculty of Pharmacy, Pharmacy Chabib, Lutfi; Universitas Islam Indonesia Hamzah, Hasyrul; Universitas Muhammadiyah Kalimantan Timur Permana, Andi Dian; Hasanuddin University Faculty of Pharmacy, Pharmacy

Development of solid lipid nanoparticles loaded polymeric hydrogels containing antioxidant and photoprotective bioactive compounds of Safflower (*Carthamus tinctorius* L.) for improved skin delivery

*Nuur Aanisah[1], Sulistiawati Sulistiawati[2], Yulia Yusrini Djabir[2], Rangga Meidianto Asri[2], Sumarheni Sumarheni[2], Lutfi Chabib[3], Hasyrul Hamzah[4], Andi Dian Permana[2]**

1. Department of Pharmacy, Faculty of Science, Tadulako University, Palu 94118, Indonesia
2. Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia
3. Department of Pharmacy, Universitas Islam Indonesia, Yogyakarta 55584, Indonesia
4. Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda, 75124, Indonesia

*Correspondence should be addressed to Andi Dian Permana (Faculty of Pharmacy, Hasanuddin University, Indonesia). Email: andi.dian.permana@farmasi.unhas.ac.id

ABSTRACT

Safflower (*Carthamus tinctorius* L.) is a potent natural antioxidant because of active compounds such as quercetin (QU) and luteolin (LU). These components prevent damage to the skin caused by free radicals from UV rays. However, due to the poor solubility and transdermal permeation, the effectiveness of the compounds in showing their activity was limited. In this study, we develop solid lipid nanoparticles (SLNs)-based hydrogel formulations to enhance the solubility and penetration of two bioactive compounds found in safflower petal extract (SPE). The hot emulsification-ultrasonication method was used to produce SLNs, and to obtain high antioxidant activity, 100% v/v ethanol was used in the extraction procedure. The results showed that this approach could encapsulate > 80% of both QU and LU. Moreover, FTIR, DSC, and PXRD spectra indicated that most of the QU and LU were trapped in a lipid matrix and dispersed homogeneously at the molecular level, increasing the solubility. Additionally, SLNs-hydrogel composites are able to release two lipophilic bioactive compounds for 24 hours, which also demonstrated increased skin retention and penetrability of the QU and LU up to 19-folds. *In vitro* blood biocompatibility showed that no hemolytic toxicity was observed below 500 µg/mL. Accordingly, the formulation was considered safe for use. Sun Protective Factor (SPF) test show a value above 15, showing the excellent promising application as the photoprotective agent to prevent symptoms associated with photo-induced skin aging.

KEYWORDS: Safflower (*Carthamus tinctorius* L.); solid lipid nanoparticles; hydrogel; antioxidant; photoprotective

1. INTRODUCTION

Excessive exposure to UV radiation poses risks to skin health, even though ultraviolet rays from the sun may positively mediate vitamin D synthesis[1] and treat several conditions such as psoriasis.[2] The radiation causes acute erythema in subjects with light skin [3] and pigment changes in those with dark skin.[4] These temporary effects may predispose one to the chronic effects of UV radiation, including photoaging, immunosuppression, and photocarcinogenesis.[5–7] There are many endogenous oxidative stress reduction mechanisms present in the human body. Antioxidants are believed to help increase resistance to oxidative stress and reduce indications of photo-induced skin aging. In recent years, natural antioxidants have been an alternative to replace synthetic ones, which are widely suspected of having toxic effects and promoting carcinogenesis. Some well-known antioxidants are polyphenols and flavonoids because they have phenolic OH groups, which allow them to chelate highly redox-active metal ions and enhance their protective effect against oxidative stress.[8,9]

Safflower is a dried stigma of the petals of *Carthamus tinctorius* L. and is classified as a potent natural antioxidant. Through several studies, it has been reported to have antioxidant activity in preventing/treating age-related diseases and oxidative stress such as neuronal[10] and cardiovascular disorders[11,12] as well as cancer.[13,14] This is presumably because it contains chemical compounds such as quercetin (QU)[15,16] and luteolin (LU).[17,18] Due to their potential to contribute a hydrogen atom to the DPPH radical, these bioactive components demonstrated the synergistic impact of a high radical scavenging activity. However, there are a few limitations caused by the original nature, such as low solubility in water resulting in reduced skin permeation abilities.[19–22] In addition, the stratum corneum, which is the outermost layer of the skin, acts as a penetration barrier by blocking the delivery of active ingredients into the skin. This is important because the potential efficacy of QU and LU in preventing cell damage until necrosis mainly occurs in the epidermis.[23,24] Various approaches in terms of drug targeting

and carrier systems with different routes of administration were studied to overcome these limitations and achieve more effective therapeutic efficacy.[25–29]

Solid lipid nanoparticles (SLNs) has many features that are advantageous for topical application. It was found that this approach has been a promising carrier for photoprotective agents such as silymarin,[30] tocopherol acetate,[31] and *Aloe vera* powders.[32] SLNs seem to be the main determinant of the extent of lipophilic compounds to penetrate into the epidermal layer of the skin after topical application. Owing to their small size and larger surface area, it is beneficial for targeting skin and upper layers of epidermis to exert its activity in protecting skin tissue from oxidative stress and photoaging. Furthermore, the lipophilic active ingredient trapped between solid lipids offers several benefits. They are physically stable due to a solid matrix, can effectively encapsulate drug molecules, and increase their stability and the penetration of the active substance into the epidermal layer of the skin after topical application, resulting in increased bioavailability and greater efficacy.[33–35] In addition, SLNs have an occlusive effect, which can be used to increase the skin's moisture for topical application.[36]

Solid lipid nanoparticles (SLNs) are systems with aqueous colloidal dispersions that have a matrix with solid lipids instead of oil. Several types of solid lipids have been used to prepare SLNs [37–39] but Geleol® (glycerol monostearate) is a fascinating since even at low concentrations is enough to produce SLNs that, after cooling, have a semisolid appearance.[38,39] This occurred due to the lower melting point and composition of Geleol® which contains high amount of monoglycerides, that can form hydrogen bonds with water molecules to promote swelling and exhibit a semisolid appearance.[39]

Most sun care products are semisolid in the form of ointments, gels, cream, or lotion forms and normally preferred because of their more convenient application. Tursili et al. found that the hydrogel carrier provided stabilization of the microparticle-entrapped sunscreen and increased the skin's retention capacity compared to other carriers.[40] Therefore, hydrogels have

received much attention due to their unique advantages: a three-dimensional pore structure that fits the extracellular matrix.[41–43]

However, few studies have examined the skin delivery of bioactive compounds of safflower which has the lipophilic properties as the antioxidant and photoprotective bioactive compounds. Therefore, in our study we investigated the increased penetration of antioxidant substances of safflower petals extract (SPE), QU, and LU, loaded into a solid lipid nanoparticulate-based hydrogel formulation. The extraction solvent selection was optimized to achieve the maximum phenolic and flavonoid content, and the total phenolic (TPC), flavonoid (TFC), and antioxidant activity were investigated. Subsequently, the SPE-loaded SLNs were formulated, optimized, and characterized. Solubility studies, *in vitro* release and the haemolytic assay were conducted. Finally, considering that antioxidants can efficiently adhere to the skin surface for topical delivery, a hydrogel dosage form was used to apply and hold SPE-loaded SLNs on the skin for protection against UV radiation.

2. EXPERIMENTAL SECTIONS

2.2.1. Materials

The safflower samples were obtained from Bone, South Sulawesi, Indonesia. Geleol® (glycerol monostearate) was generously given by Gattefosse Pvt. Ltd., France. Carbomer, poly(vinyl alcohol) PVA (9–10 kDa), Tween80 and sodium dihydrogen phosphate were purchased from Sigma-Aldrich (Dorset, UK). Other chemicals used in this experiment were analytical grade.

2.2.2. Extraction Process

The extraction process, the determination of total phenolic content, the determination of total flavonoid content, the antioxidant activity determination and HPLC analysis are shown in the supplementary materials in Section S2.

2.2.3. Formulation of SPE-loaded SLNs. SLNs containing SPE were produced through an emulsification-solvent evaporation method. Several formulas were prepared using various lipid concentrations (Geleol®) duration of homogenization, and type of surfactants (Tween 80 and PVA) to determine the effect of these formulation parameters on the physicochemical properties of obtained SPE-loaded SLNs. These various formulation parameters are presented in Table 1.

Table 1. Formulation parameters used to prepare SPE-loaded SLNs

Formulation	Geleol® (mg)	Tween 80	PVA
F1	100	1%	-
F2	150	1%	-
F3	200	1%	-
F4	250	1%	-
F5	100	-	1%
F6	150	-	1%
F7	200	-	1%
F8	250	-	1%
F9	100	2%	-
F10	150	2%	-
F11	200	2%	-
F12	250	2%	-
F13	100	-	2%
F14	150	-	2%
F15	200	-	2%
F16	250	-	2%

In this study, 100 mg of SPE and Geleol®, which were accurately weighed according to Table 1 were dissolved in 15 mL of chloroform:methanol mixture (1:1 v/v). The organic phase was slowly poured into the aqueous phase containing the surfactant and processed with a homogenizer Ultra-Turax IKA® T18 (IKA, Campinas, Brazil) at 15,000 rpm for 10 minutes for the F1-F8 formula and 20 minutes for the F9-F16 formulations. Afterward, the organic solvent was removed by stirring at 4 h at room temperature in a fume hood.

2.2.4. Characterization of SLNs

2.2.4.1. Particle Size, PDI, and Zeta Potential. The assessment of mean particle size, polydispersity index (PDI), and zeta potential of the SPE-loaded SLNs was conducted by dynamic light scattering at 90° scattering angel using using a Malvern Zeta Sizer® (Malvern Instruments Ltd, UK), at 25 °C. Specifically, Z-average (d.nm) with intensity (%) was used to represent the particle size. Before the measurements, the SLNs were diluted with distilled water.

2.2.4.2. Encapsulation efficiency. The encapsulation efficiency (EE) was determined by measuring the free drug concentration with the centrifugation method. Dispersion SPE-loaded SLN was centrifuged in a high-speed centrifuge at 14,800 × g for 60 min at 4 °C. Then, the supernatant as the free drug concentration was determined using the previously described HPLC method. The percentage of EE was calculated according to the following equation.[44,45]

$$\%EE = \frac{a-b}{a} \times 100\% \quad (1)$$

where a and b are the amounts of total SPE-loaded SLN used in the formulation and free drug in the supernatant, respectively; their units are μg/mL.

2.2.4.3. FTIR study. The SLNs formulation was subjected to FTIR (Fourier Transform Infrared) Spectrometer Accutrac FT/IR-4100 Series (Jasco, Essex, UK) connected to Diamond MIRacle™ ATR over a wavenumber range of 4000–400 cm^{-1}.

2.2.4.4. DSC. The DSC analysis was carried out using TA Instruments DSC Q100 (TA Instruments, New Castle, Delaware, USA) to obtain thermograms of SPE, blank SLN, and SPE-loaded SLNs. The specific amounts of each sample were heated in an aluminum pan at a heating rate of 10 °C/min from 25 to 280℃ under a nitrogen atmosphere which was purged at a flow rate of 10 mL/min. The results obtained from the DSC analysis were analyzed using TA Instruments Universal Analysis, version 4.5A.

2.2.4.5. Powder X-ray diffraction (PXRD). Crystalline characteristics of the SLNs formulation were assessed through PXRD-X Miniflex™ (Rigaku Corporation, Tokyo, Japan). Studies were carried out on the samples by exposing them to Ni-filtered radiation, Cu Kβ (15 mA, 30 kV).[46]

2.2.4.6. Scanning Electron Microscope. Morphological examination of SPE-loaded SLN was examined using a scanning electron microscope (SEM) (JEM-1400Plus, Tokyo, Japan). Initially, 100 µL of SLNs were air-dried and coated with gold under vacuum sputter. The analysis was carried out at 15 kV.

2.2.4.7. Solubility analysis. The saturated solubility analysis of quercetin (QU) and luteolin (LU) in SPE and SPE-loaded SLNs was performed in 20 mL of water or n-octanol in a closed glass vial at room temperature. The blend was mixed under stirring at 500 rpm for 1 h. Afterward, the blend was centrifuged at 2800 × g for 15 min. Subsequently, the supernatant was obtained and analyzed for solubility after appropriate dilution using HPLC analysis.[46]

2.2.4.8. In vitro haemolytic assay. *In vitro* haemolytic activity studies were carried out to determine the safety and biocompatibility of SLNs formulations loaded with SPE. Wistar rat's erythrocyte samples were separated from plasma using centrifugation at 2000 rpm (20 minutes). Then the erythrocytes were washed with PBS 3 times, mixed using a vortex mixer and centrifugated for 10 min at 2000 rpm. Then the washed erythrocytes were resuspended with PBS until they reached a concentration of 10% v/v. Afterward, 100 µL of tested samples were mixed with 900 µL of erythrocytes. The mixture was then incubated for 1 h at 37 °C, centrifuged for 10 min at 7000 rpm, and the absorbance of the supernatant was detected using a UV–Vis Spectrophotometer (Shimadzu Co., Ltd., Tokyo, Japan) at 540 nm. As a positive and negative control, PBS and water were also measured, respectively. The percentage of haemolytic activity was calculated through the equation below [47,48]:

$$\text{Hemolysis (\%)} = \left(\frac{Abs_{test\ sample} - Abs_{negative\ control}}{Abs_{test\ sample} - Abs_{negative\ control}}\right) \times 100\% \quad (2)$$

2.2.4.9. Stability study. Dispersion of SPE-loaded SLN was filled into sealed glass vials and stored in conditional room temperature (25 ± 2°C/60 ± 5% Relative Humidity) for 1 month. Every week, the formulations of SPE-loaded SLN were assessed in terms of particle size.

2.2.4.10. In vitro release study using mathematical modelling. *In vitro* release studies were conducted by dialysis membrane diffusion technique. Briefly, the dispersion was sealed into Spectra-Por®, a 12,000-14,000 MWCO dialysis membrane bag (Spectrum Medical Industries, Los Angeles, CA, USA). To achieve sink condition during the release study, PBS (pH 7.4) containing 1% Tween 80 was used as a medium. The study was carried out at 37 °C in an orbital shaker (100 rpm). Furthermore, at each interval time, 1 mL of medium was collected from the PBS and replaced with the same volume of the fresh PBS. Samples were filtered and quantified by the HPLC methodology described in section 2.2.1.6.

To find out the drug release model from the manufactured dosage form, the percentage of drug released was then applied in various mathematical models as follows:

$$\text{Zero order: } Q_t = Q_0 + K_0\, t \tag{3}$$

$$\text{First order: } \ln Q_t = \ln Q_0 + K_1\, t \tag{4}$$

$$\text{Higuchi: } Q_t = K_H\sqrt{t} \tag{5}$$

$$\text{Korsmeyer-Peppas: } Q_t = K_t\, n \tag{6}$$

$$\text{Hixson Crowell: } Q_0^{1/3} - Q_t^{1/3} = K_s t \tag{7}$$

where Q_t (%) represents the amount of compound released at time t, Q_0 represents the initial amount of Q_t, t represents the time, n represents the diffusion release exponent, K_0, K_1, K_H, K_t and K_s represent the release coefficients according to relevant kinetic models.

2.2.5. Preparation of Hydrogel containing SLNs. To prepare SLNs hydrogel, Carbomer 940 (1% w/w) and glycerol (10% w/w) were mixed in distilled water and hydrated at room temperature overnight. The dispersion was neutralized by adding triethanolamine (1% w/w), producing a clear hydrogel. Meanwhile, the prepared SPE-loaded SLNs (1% w/w) were incorporated into the hydrogel and mixed evenly at 1000 rpm using a homogenizer. The blank hydrogel was prepared as before but without SPE being loaded into the SLNs.

2.2.6. Evaluation of hydrogel

2.2.6.1.Drug Uniformity Content and pH. Briefly, 0.5 g of SLNs-based hydrogel was dispersed in 5 mL of methanol and then sonicated for 30 min in a sonicator bath. Following this, the mixture was centrifuged for 15 min at 7000 rpm, and the concentration of bioactive compounds was analyzed using HPLC. Meanwhile, the apparent pH value of hydrogel formulation was measured using a pH meter under 25 ± 1 °C.

2.2.6.2. Spreadability. The spreadability was determined by observing the spreading diameter of 0.5 g of hydrogel after being tied up with a standardized weight of 500 g on the upper plate for 5 min.

2.2.6.3. In Vitro Skin Occlusivity Evaluation. The *in vitro* occlusion test was conducted according to the De Vringer method with slight modification.[49] Briefly, the first 100 mL beaker was filled with 50 mL of water and covered with Whatman filter paper (Whatman number 6, cutoff size: 3 µm, USA). Furthermore, 250 mg of SPE-loaded SLN hydrogel was spread on the surface of filter paper and stored at 32±0.5 °C for 48h (skin temperature). To calculate water loss from evaporation, samples were weighed at 0, 6, 24, and 48 h. Furthermore, for reference, the measuring cup was covered with filter paper but without the application of the hydrogel. The occlusivity (F0) was calculated as:

$$F0 = \frac{W0 - W1}{W0} \times 100 \quad (8)$$

where W0 is the amount of water loss of the reference and W1 is the amount of water loss of the formulation group.

2.2.6.4. Ex-vivo Bioadhesive Strength. The bioadhesive strength of the blank (negative control) and SPE-loaded SLN hydrogel formed (positive control) was measured *ex vivo* using the skin tissue of Wistar rats using the modified balance method. The left side of the double-pan scale was removed, a glass bottle was hung upside down by the string, and another glass bottle was placed under it. Then, the rat skin tissue was washed using normal saline (0.9% NaCl w/v), after which it was followed by immersion in phosphate buffer pH 7.4, which was maintained at 37 ± 1 °C for 30 minutes. Furthermore, the prepared skin tissue was attached to the upper and lower vials, and each 1 g of the hydrogel formulation was pipetted between the skin tissue, which was cut to 4.5 cm^2. The right pan of a double-pan was added with loads every 30 seconds over the pan to measure the maximum weight required to detach the hydrogel from the skin tissue. The bioadhesive strength was calculated in terms of force per unit area using the following equation [47,48]:

$$\text{Bioadhesive strength } (N/m^2) = \frac{m \times g \times 0.1}{A} \quad (9)$$

where m is the weight (grams) required to remove the formed hydrogel from the rat skin, g is gravity acceleration (9.8 m/s^2), and A is the surface area of the rat skin (cm^2).

2.2.6.5. SEM analysis of hydrogel. SEM analysis was carried out to observe the surface morphology of SLNs-based hydrogel. The hydrogel was mounted and sputter coated with gold after being lyophilized under vacuum. Then, SEM analysis of was carried out scanning electron microscope (SEM) (JEM-1400Plus, Tokyo, Japan) with an accelerating voltage of 20 kV.

2.2.6.6. Ex-vivo Skin Permeation and Retention Studies. *Ex-vivo* skin permeation of SPE (negative control) and SPE-loaded SLNs formulation (positive control) was conducted using the Franz-type diffusion cells. The abdominal skin of Wistar rats was shaved and mounted on 25 mL Franz-type diffusion cells after being equilibrated in PBS (pH 7.4) containing 1% w/v Tween 80 as a medium with an effective diffusion area of 4.9 cm^2. The collection of the skin was approved by Ethical Committee, Hasanuddin University, Indonesia. Furthermore, the receptor chamber was filled with a 10 mL diffusion medium, and the cells were maintained at (37 ± 0.5 °C) with stirring at 100 rpm. The 0.5 g SLNs-based hydrogel containing SPE was applied to the skin surface, and a 0.5 mL sample of the receptor chamber was withdrawn at predetermined time intervals. Afterward, it was immediately replaced with an equivalent volume of fresh medium maintained at 37 ± 0.5 °C. The samples were filtered through an aqueous 0.45 µm membrane filter and analyzed by HPLC to determine the amount of drug permeated from SLNs-based hydrogel.

The drug retained in the skin was also estimated after 24 h following the skin permeation study. The skin was removed and cut into small pieces using scissors, and the pieces were washed three times with distilled water to remove any excess formulations. It was subjected to bath sonication for 6 h to extract the drug from the skin. The samples were centrifuged at 2800 ×g for 15 min, and the supernatant was collected and subjected to HPLC for analysis.

2.2.6.7. Antioxidant Activity and Sun Protective Factor (SPF) of Optimised Formulation. The antioxidant activity of optimized hydrogel SPE-loaded SLNs was investigated using the previously described method for SPE. Determination of *in vitro* SPF of SPE using the UV-Visible spectrophotometric method. A quantitative solution with the range concentration of 0.1-1 mg/mL were prepared. The SPF value was determined by calculating the absorption of each solution at a wavelength of 290-320 nm with an interval of 5 nm. SPF values were calculated as follows:

$$SPF = CF \times \Sigma_{290\ nm}^{320\ nm} EE_{\lambda} \times I_{\lambda} \times Abs_{\lambda} \quad (10)$$

where CF (Correction Factor) is 10 (constant), EE_{λ} is the erythema effect spectrum, I_{λ} is the sun intensity spectrum, and Abs is the absorbance of the analyzed sunscreen product.

2.2.7. Statistical Analysis. Quantitative data were presented as mean ± standard deviation (SD) for three replicates. GraphPad Prism® version 6 (GraphPad Software, USA) was used to perform statistical analysis of the data obtained, and significant differences were expressed in p values < 0.05.

3. Results and discussion

This study was conducted to study the antioxidant potential of SPE-loaded SLN in topical drug delivery. The results of the extraction process, the determination of total phenolic content, the determination of total flavonoid content, the antioxidant activity determination and HPLC analysis are depicted in the supplementary materials in Section S3. In order to enhance the penetration of antioxidant substances of safflower petals extract (SPE) such as QU and LU, SPE-loaded SLN formulations have been proposed in the present paper.

3.1 Solid Lipid Nanoparticle Formulation. The effects of various parameters were studied in optimizing SPE-loaded SLNs formulation, such as difference in Geleol® concentration, duration of homogenization, and type of surfactants (Tween 80 and PVA (M_w=9-10 kDa)) used. Based on previous research, Geleol® was selected because it has the lowest melting point of ~55 °C. This could lead to the lowest viscosity in the medium, resulting in increased homogenization and sonication efficiency to produce smaller particles.[50] Additionally, it showed a semisolid appearance after cooling, even in low concentrations.[39] In the screening of surfactants, Tween 80 and PVA were used because of their non-ionic nature.[39,51]

3.2 Characterization of SPE-loaded SLNs

3.2.1. Particle size, PDI, Zeta Potential, and Encapsulation efficiency

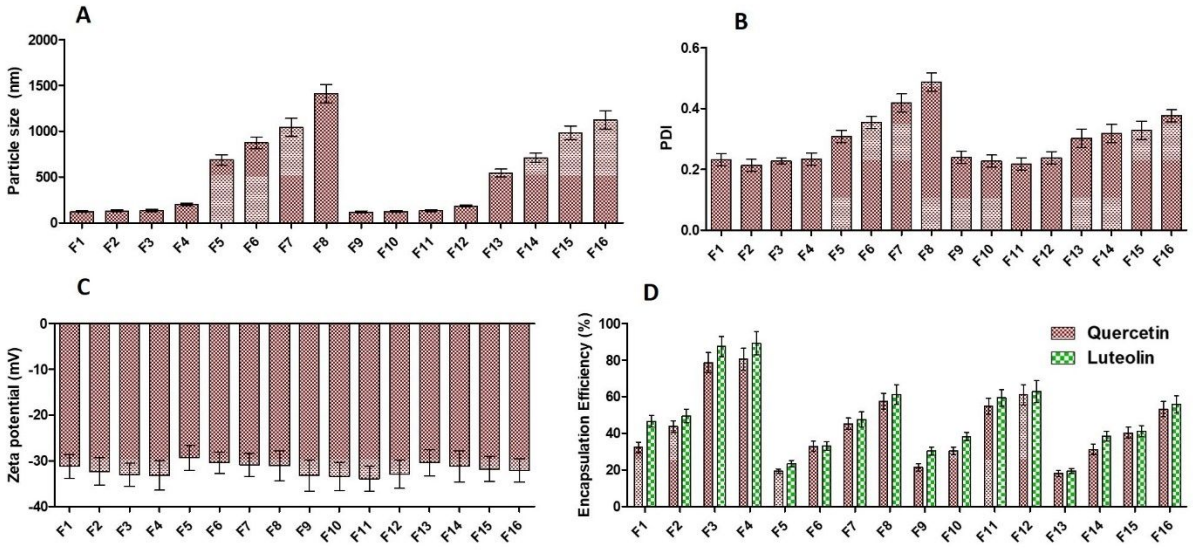


Figure 1. Characterization of nanoparticles. Particle size (A), PDI (B), Zeta potential (C) of different SPE-loaded SLNs formulations, and Encapsulation efficiency of QU and LU (D) of different SPE-loaded SLNs formulations (means ± SD, n = 3)

As shown in Figure 1A-B, the high concentration of Geleol in the SLNs formulation increased the particle size and PDI of SLNs only for the PVA surfactant case. This can be caused by increasing the concentration of Geleol will increase the viscosity of the dispersion. Therefore, the efficiency of the homogenization and sonication steps in reducing the particle

size of the melted lipid droplets was reduced. Meanwhile, Tween 80 surfactant seemed to produce a more consistent particle size and PDI regardless of Geleol concentration. Regarding the zeta potential measurement, the SLNs varied between -29.33 and -33.91 mV (Figure 1C), indicating electrostatic repulsion between particles to prevent aggregation and stabilize the dispersion of SLNs. [52] All formulations had a negative charge due to the anionic nature of the lipids. The narrow range of zeta potentials could be related to the use of same lipid types with different concentrations. Moreover, the high concentration of Geleol in the SLNs formulation also increased the encapsulation efficiency (EE) of QU and LU due to the solubilization of active compounds in the lipid (Figure 1D).

Regarding the type of surfactant utilized in the SLNs formulation, Tween 80 (HLB: 15) and PVA (HLB: 18) are both non-ionic surfactants. In the same concentration, the surfactant Tween 80 was preferred over PVA because it showed a decrease in particle size and increased encapsulation efficiency (EE) of QU and LU. The particle size of SPE-loaded SLNs prepared with higher concentrations of Tween 80 were seemed beneficial to slightly reduce the particle size. Besides, it also led to lower the PDI values. Tween 80 has an HLB value in the effective range of 12-16 to produce a stable oil-in-water (O/W) emulsion. A high %EE can also be attributed to the lipophilic nature of QU and LU due to the higher affinity for the lipid matrix. Consequently, it can increase the number of drugs encapsulated in the lipid core.

Increasing the homogenization and sonication time from 10 to 20 minutes decreased the particle size significantly ($p < 0.05$). The prolonged homogenization and sonication time results in more energy for the nanoparticle dispersion, reducing the size of the SLNs. Considering that the encapsulation efficiency decreased with increasing homogenization time, 10 minutes was sufficient to obtain the optimal formulation of SPE-loaded SLNs.

According to the results of particle size and encapsulation efficiency of both QU and LU compounds, F3 and F4 were selected to be the optimal formula. The results obtained from F3 and F4 were not statistically significant compared to one another.

3.2.2. FTIR Study. Figure 2A describes the FTIR spectrum of SPE and its formulation in SLNs. The specific functional groups QU and LU in the FTIR spectrum are summarized as follows: SPE: 1150-1500 cm^{-1} (aromatic bonding), 1239 cm^{-1} (-C-O-C bonding), 1367 cm^{-1} (-C-O–H, stretching), 1521 cm^{-1} (C-C=C asymmetric stretching), 1617 cm^{-1} (-C-O stretching of quercetin and C=O vibration of the central heterocyclic ring of luteolin), 1681 cm^{-1} (C=O stretching of the ketone carbonyl), 3434 cm^{-1} (-OH vibration of the phenolic group of QU and LU). Furthermore, in the spectrum of Geleol, specific peaks were observed at 1215 cm^{-1} and 721 cm^{-1}, corresponding to C-H stretching and C-H bending, respectively. The results showed that the functional groups characteristic of QU and LU were still recognizable in the FTIR spectrum of the physical mixture and the SPE-loaded SLNs formulation. This indicates an adequate entrapment of QU and LU in a lipid matrix and no chemical interaction between the drug and excipients used in the formulation.

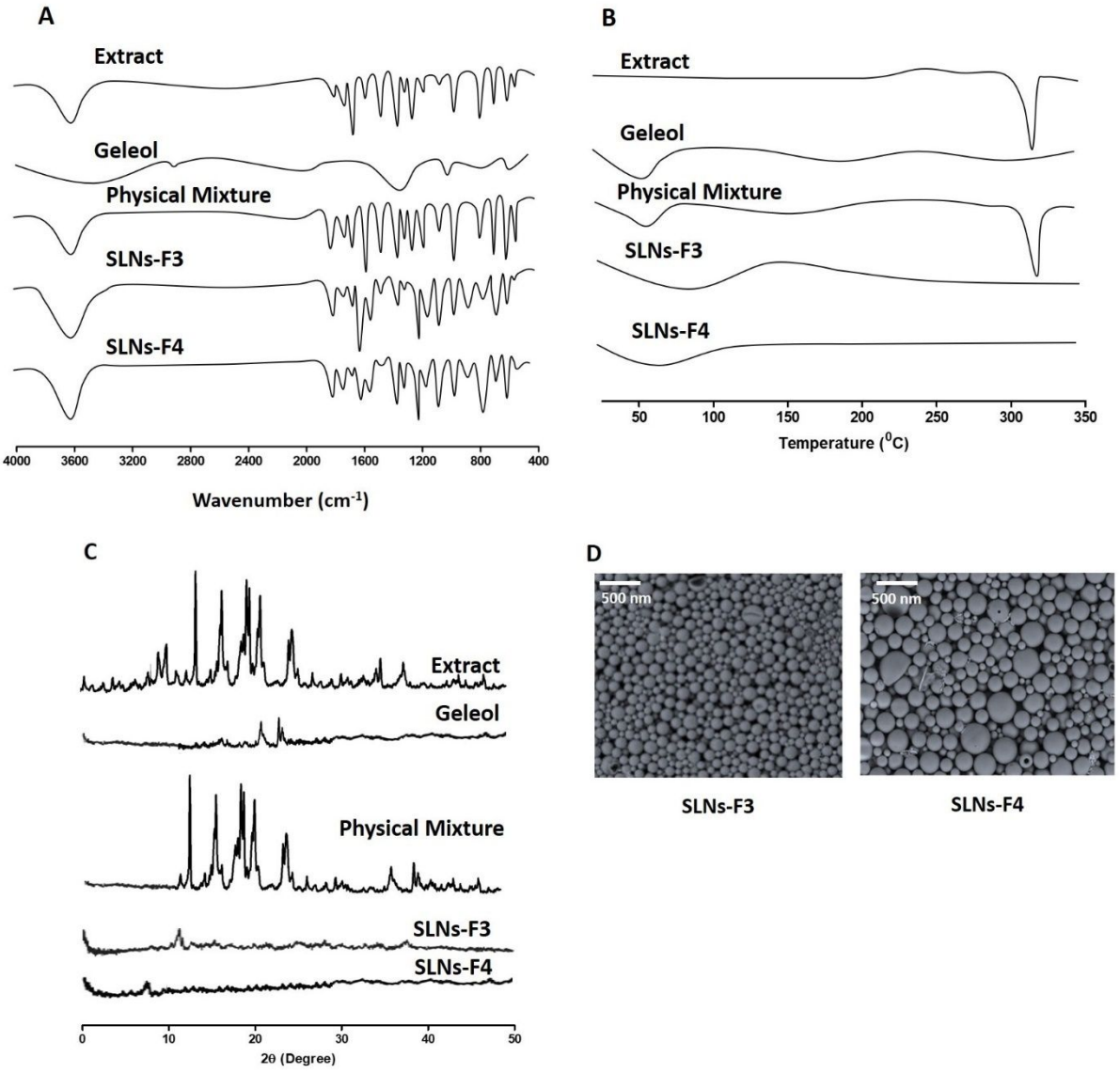


Figure 2. FTIR spectra (A), DSC (B), PXRD diffraction (C) of extract, Geleol, physical mixture, F3 and F4 SPE-loaded SLNs formulation, SEM images of F3 and F4 SPE-loaded SLNs formulation (D)

3.2.3. ***DSC analysis.*** Several studies regarding the thermal analysis of SLNs used variations of the scanning rate from 1-50 ml/min under nitrogen purge [53–55]. This scan rate level will give different transition temperatures and peak shapes on the DSC melting curve. At high scanning rates, there is no time for the heat to be transmitted from the heating elements of the DSC cell to the sample, resulting in a higher peak transition. Moreover, too high heating rates will give the smooth shape of the melting curve and, as a result, detailed information may get lost. Meanwhile, at a lower scanning rate it also causes a narrowing of the melting endotherms or crystallization exotherms. A low scan rate (1 °C/min) gives several peaks that are close together. Thus, in the current study we used 10 °C/min to produce a distinguishable thermal transition [56].

DSC thermograms of SPE and SPE-loaded SLN are shown in Figure 2B. The profile of the extract showed a sharp endothermic peak at 321 °C, which corresponds to QU and LU melting points that show a highly crystalline nature for these two materials.[57,58] The peak was found at 61 °C in the Geleol thermogram, indicating its melting point. In the thermogram of the physical mixture, all peaks were also observed. However, this peak disappeared in the SPE-loaded SLNs formulation. This may indicate complete encapsulation of the two compounds or the transformation to an amorphous state molecularly dispersed in the lipid matrix.[51] The nanoparticles show glass transition characteristics that occurred between 30-80 °C, as compared to the extract thermogram. The appearance of the glass transition and the decrease in melting temperature might be attributed to the reduced particle size and increased surface area (Gibbs–Thompson effect).[59]

3.2.4. PXRD and Scanning Electron Microscope. Figure 2C represents the XRD pattern for the SPE and the optimized formulation, such as F3 and F4. The XRD SPE pattern showed intense peaks at an angle of 2Θ 15-30°, and the crystalline nature of the extract compounds was found. XRD pattern of Geleol showed low peaks between 20-25°, indicating its low crystallinity. In the physical mixture diffractogram, all peaks were identified. Meanwhile, the peak disappeared in the XRD spectrum of SPE-loaded SLN, which showed amorphous nature that increase solubility. Formulation amorphization revealed that most of the drug was trapped in lipids and dispersed homogeneously at the molecular level, in agreement with the previous DSC results.

SEM images of the optimized SLNs (F3 and F4) are presented in Figure 2D. The results exhibited spherical and homogeneous particles, and the SEM photograph suggested that the SLN possessed a smooth surface. The particle size value shown in the SEM was in close agreement with the size measurement by PSA, which is approximately 135.98 ± 12.09 nm and 202.36 ± 14.21 nm for F3 and F4, respectively.

3.2.5. ***Solubility Analysis.*** The solubility of QU and LU is one of the essential factors that must be considered in the SLN formulation. In this study, we compared the solubility of QU and LU from SPE, as well as from formulas F3 and F4 SPE-loaded SLN in water and n-octanol solvents. The test results are depicted in Table 2, which shows that the water solubility of the compounds QU and LU (SPE) was observed to be 28.32 ± 2.13 μg/mL and 17.23 ± 1.61 μg/mL, respectively. Meanwhile, the solubility of QU and LU (SPE) compounds in n-octanol showed a significantly different solubility effect ($p < 0.05$) compared to their solubility in water, namely 498.56 ± 43.21 μg/mL and 504.34 ± 47.87 μg/mL, for QU and LU, respectively. This also explains the hydrophobic nature of the compound.

Table 2. Solubility of QU and LU from Safflower petals extract (SPE), F3, and F4 of SLN formulations (means ± SD, n = 3).

Compound	Samples	Aqueous solubility (μg/mL)	n-octanol solubility (μg/mL)
Quercetin (QU)	SPE	28.32 ± 2.13	498.56 ± 43.21
	SLNs-F3	385.43 ± 21.43	509.44 ± 47.03
	SLNs-F4	309.41 ± 29.37	503.12 ± 48.38
Luteolin (LU)	SPE	17.23 ± 1.61	504.34 ± 47.87
	SLNs-F3	358.43 ± 31.98	598.57 ± 54.39
	SLNs-F4	299.73 ± 27.46	549.59 ± 51.37

Furthermore, the solubility of QU and LU of the SPE-loaded SLN formulation showed an increase in the solubility of QU and LU in water and n-octanol. The solubility of QU and LU of the SPE-loaded SLN formulation in water showed a significant ($p < 0.05$) increase of approximately 12-fold and 20-fold for QU and LU, respectively, compared to the solubility of the compound from SPE in water. A small increase was observed in n-octanol, approximately 1.1 times higher than SPE. This significant increase ($p < 0.05$) of aqueous solubility was due to the heating step of the hot emulsification process, in which the active compounds partitioned from the lipid droplets in the SLN formulation melted into the aqueous phase.[24] Several studies have demonstrated the drug delivery capability of solid lipid nanoparticle systems in increasing solubility for hydrophobic drugs or having low solubility in water.[23,60,61]

3.2.6. In vitro hemolytic assay. In this work, the *in vitro* hemolysis assay was used to measure the hemocompatibility of SPE-loaded SLNs. The index value considered safe was less than 5%.[62] A hemolytic activity assay was carried out using Wistar rats. From Figure 3A and 3B, the formulation of SLNs containing SPE showed a hemolysis value of less than 5% at all tested concentrations (5-500 µg/mL) of SPE-loaded SLNs. Therefore, the prepared SPE-loaded SLNs had no hemolytic effect and were considered safe for use at the concentrations tested.

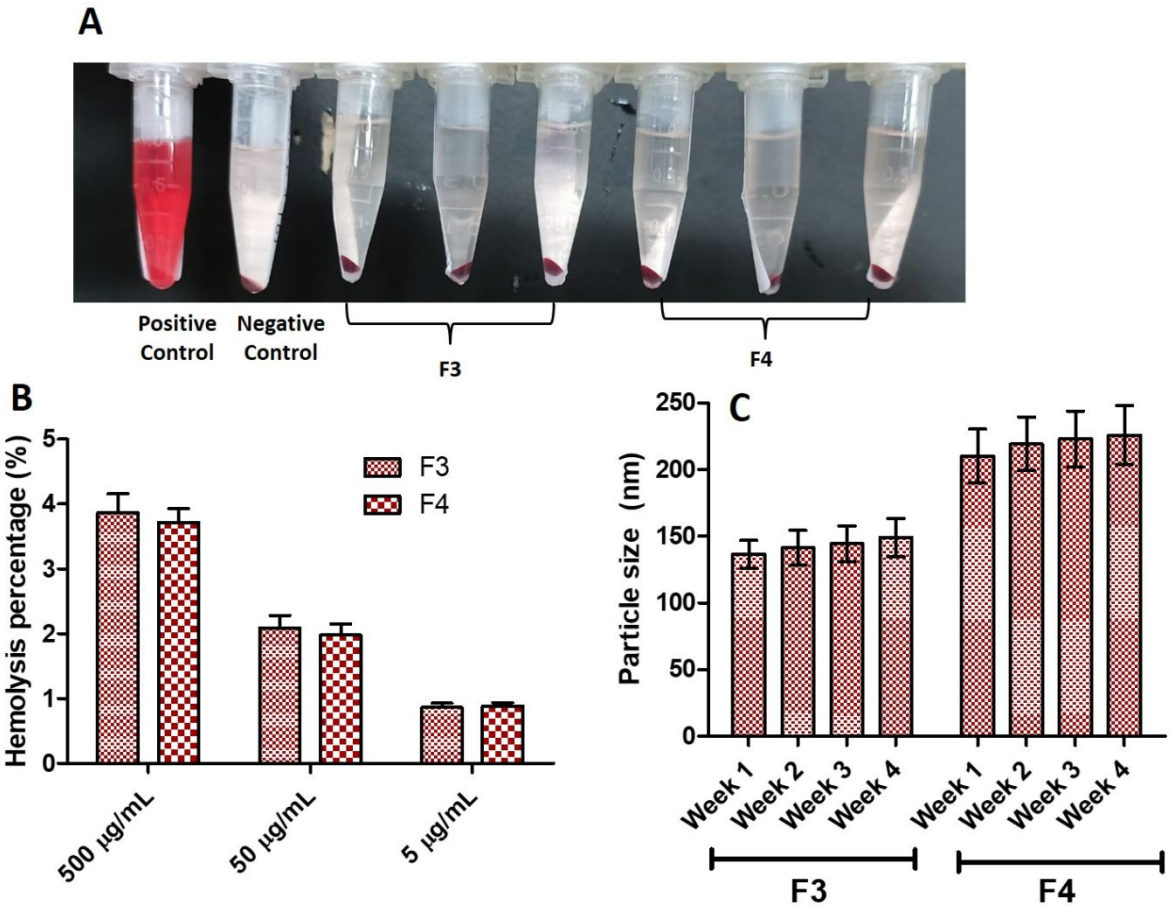


Figure 3. Hemolytic activity test. Image representative (A), hemolysis index value (B). Stability study results of SLNs (C) (Means ± SD, n = 3).

3.2.7. Stability study. In an attempt to assess the stability of the SLNs, the particle size was observed for 1 month at 25°C. The result of this study is depicted in Figure 3C, indicating that despite the increase in the particle size, there was no significant difference ($p < 0.05$) after 1 month storage. Accordingly, it could be concluded that both formulations were physically stable.

3.2.8. In vitro drug release. The cumulative percentage drug release from SPE and optimized SPE-loaded SLNs formulations were investigated for 24 h (Figure 4). The release of the active compound from SPE for 24 h was 20.35 ± 1.72% and 17.06 ± 1 .32% for QU and LU. Meanwhile, when SPE was incorporated into SLNs, the release of QU and LU became *significantly* faster ($p < 0.05$), reaching 84.98 ± 7.43% and 76.81 ± 6.43% for F3, respectively, and 73.14 ± 6.05% and 66.34 ± 5.38% for F4, respectively. Combining SLNs and hydrogels will display both component advantages such as the protection and the improvement of the solubility of the lipophilic active molecule, while improving the drug release. Even though the drug release from SPE-loaded SLN-bearing hydrogel formulations is faster, the sustained drug release for 24 hours is still be observed, suggesting their applicability as the carrier to protect the skin from the UV-rays all day.

Moreover, the percentage of drug dissolution data was fixed in several kinetic models to evaluate the dissolving mechanism and QU and LU from SLN matrices (Table 3). The most suitable release model was selected according to the correlation coefficient value. The results revealed that the release profile of the two compounds from the SLNs formulation seemed to follow the Higuchi model ($R > 0.95$). Meanwhile, the SPE-loaded SLNs showed a high correlation for the Higuchi model, where the release of the drug from the formulation was controlled by diffusion through the matrix.

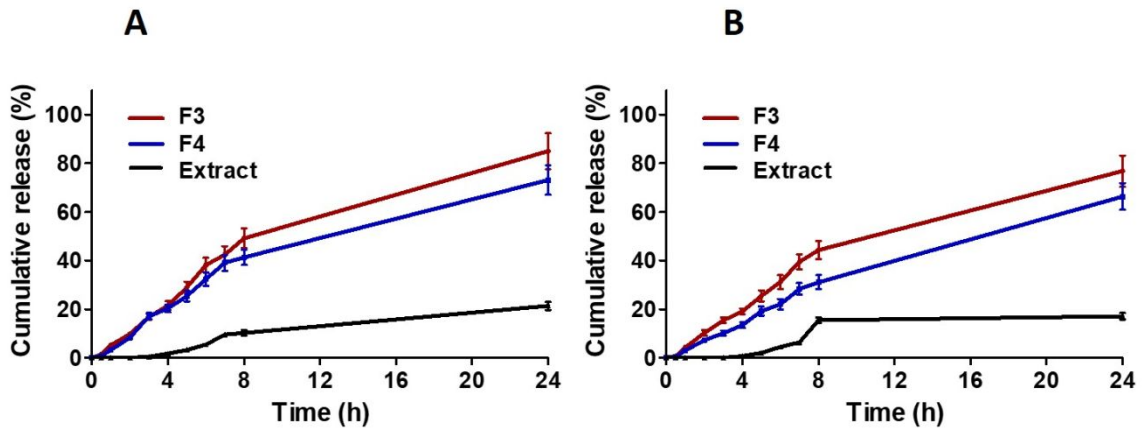


Figure 4. *In vitro* drug release profile of QU (A) and LU (B) from F3, F4, and Safflower extract (Means ± SD, n = 3).

Table 3. Kinetic models of SPE-loaded SLNs

Formula	Regression coefficient (R^2) values				
	Zero-Order	First-Order	Higuchi	Hixson-Crowell	Korsmeyer–Peppas
F3	0.81	0.93	0.98	0.87	0.95
F4	0.84	0.92	0.97	0.83	0.92

3.3 Characterization of SLNs Hydrogel

3.3.1 ***Drug uniformity and pH.*** *To* assess the effect of the formulation on the distribution of compounds in the hydrogel formulations, a content uniformity test of the two main chemicals of SPE in the SPE-loaded SLN formulation was carried out. Figure 5A showed that the analyte recovery at F3 was 97.65 ± 1.32% and 98.56 ± 1.43% for QU and LU, respectively. Meanwhile, the analyte recovery results at F4 were 99.54 ± 1.62% and 98.18 ± 1.58% for QU and LU, respectively. These results indicate that the formulation procedure carried out did not affect the recovery and homogeneity of QU and LU compounds in the hydrogel, so the two formulas produced can be considered to possess good content uniformity. Regarding formulation pH (Figure 5B), the blank hydrogel's pH was 6.74 ± 0.23, while the pH of the charged hydrogel SLNs was in the range of 5.56 ± 0.21 and 5.61 ± 0.19 for F3 and F4, respectively. The pH of the hydrogel was in the normal skin pH range of 5 to 6. The pH test was important to determine the acidity level of the formulation made to prevent irritation.

3.3.2 ***Spreadability.*** SLNs-incorporated hydrogels were further evaluated for spreadability to evaluate customer compliance, depicted in Figure 5C, and the dispersion was found to be 56.54 ± 2.87 mm for blank gel. Meanwhile, the dispersion of the F3 and F4 hydrogels was 55.98 ± 2.71 mm and 56.01 ± 2.93, respectively. The standard range of a suitable hydrogel formulation is between 50-70 mm. Based on F3 and F4, the results of the dispersion test were equally acceptable. Additionally, the incorporation of SPE-loaded SLNs into hydrogel formulation did not change ($p > 0.05$) the spreadability of the final products.

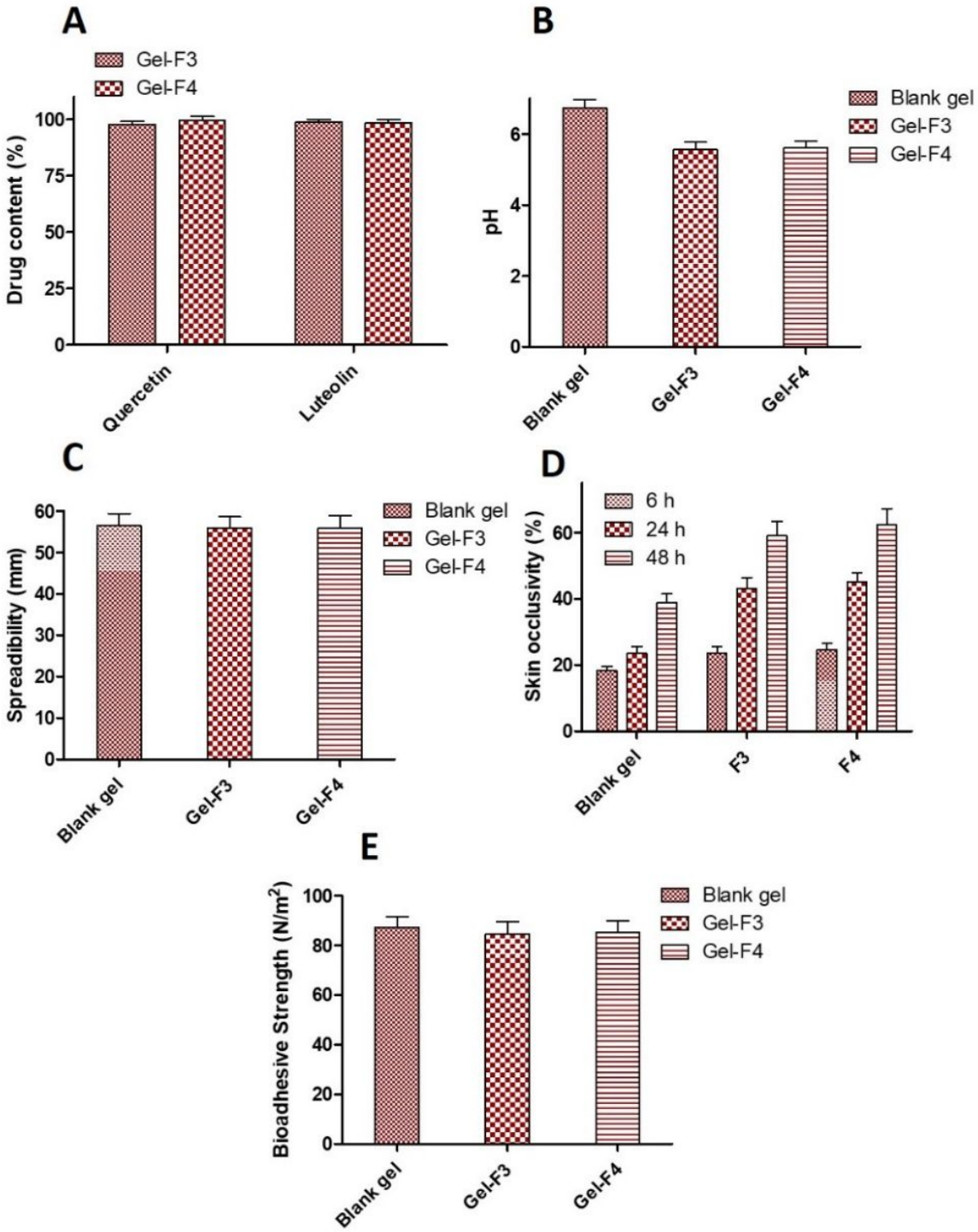


Figure 5. Drug uniformity content (A), pH (B), spreadability (C), *in vitro* skin occlusivity (D), and bioadhesive strength (E) of hydrogel formulation in comparison with blank hydrogel (means ± SD, n = 3).

3.3.3 ***Skin Occlusivity Evaluation.*** To assess the ability of the SPE-loaded SLN formulation to maintain its skin hydrating properties after application, an *in vitro* evaluation of the skin occlusives was carried out. The results of *in vitro* skin occlusives for 48 h are shown in Figure 5D. Furthermore, F3 and F4 showed an increase in occlusiveness of 59.09 ± 4.31% and 62.43 ± 4.87%, respectively, when the shell occlusive values of the blank gels (38.77 ± 2.78%) were compared with those of the hydrogel SLNs. The better occlusion properties incorporated with SPE-loaded SLNs can be attributed to the dense nature of the lipid components. This prevents the evaporation of water to a greater extent and also to the aqueous phase of the hydrogels. It can increase skin hydration for up to 48 hours, allowing drug penetration through the stratum corneum due to the reduction of the corneocyte gap.[63]

3.3.4 ***Bioadhesive Strength.*** Flexibility and softness are characteristics of an ideal topical hydrogel, but the ability to withstand pressure damage from external mechanical forces and the ability to stick to the skin for a long time after application are also characteristics that must be met by the hydrogel. Therefore, in this study, a bioadhesive strength test was carried out on the SPE-loaded SLN formula. There are three types of gel tested in this study, namely blank gel and gel containing F3 and F4. Figure 5E shows the bioadhesive strength of each hydrogel. The results of the three gels showed that the addition of SPE-loaded SLNs into the hydrogel did not change the ability of the gel to adhere to the skin. Then based on statistical results, no significant difference was observed for each hydrogel made. This particular trend can be attributed to the fact that the anionic hydrophilic polymer used, Carbopol 940, tends to disentangle due to its carboxylic acid groups being partially ionized in solution, which leads to bioadhesion.[67] In addition, Carbopol 940 undergoes crosslinking so that it has a longer contact time on the skin, so they are more available for interpenetration with the superficial epithelial cells.[68]

3.3.5 ***SEM analysis of hydrogel.*** Following the observation using SEM, it was found that all hydrogel formulations possessed rough surface with higher brawl borders. The SEM images of all formulations are exhibited in Figure 6. As shown, the SLNs were completely entrapped in the hydrogel matrices. Therefore, this could potentially provide sustained release behaviour of the bioactive compounds from the Carbopol-based hydrogel formulations.[64]

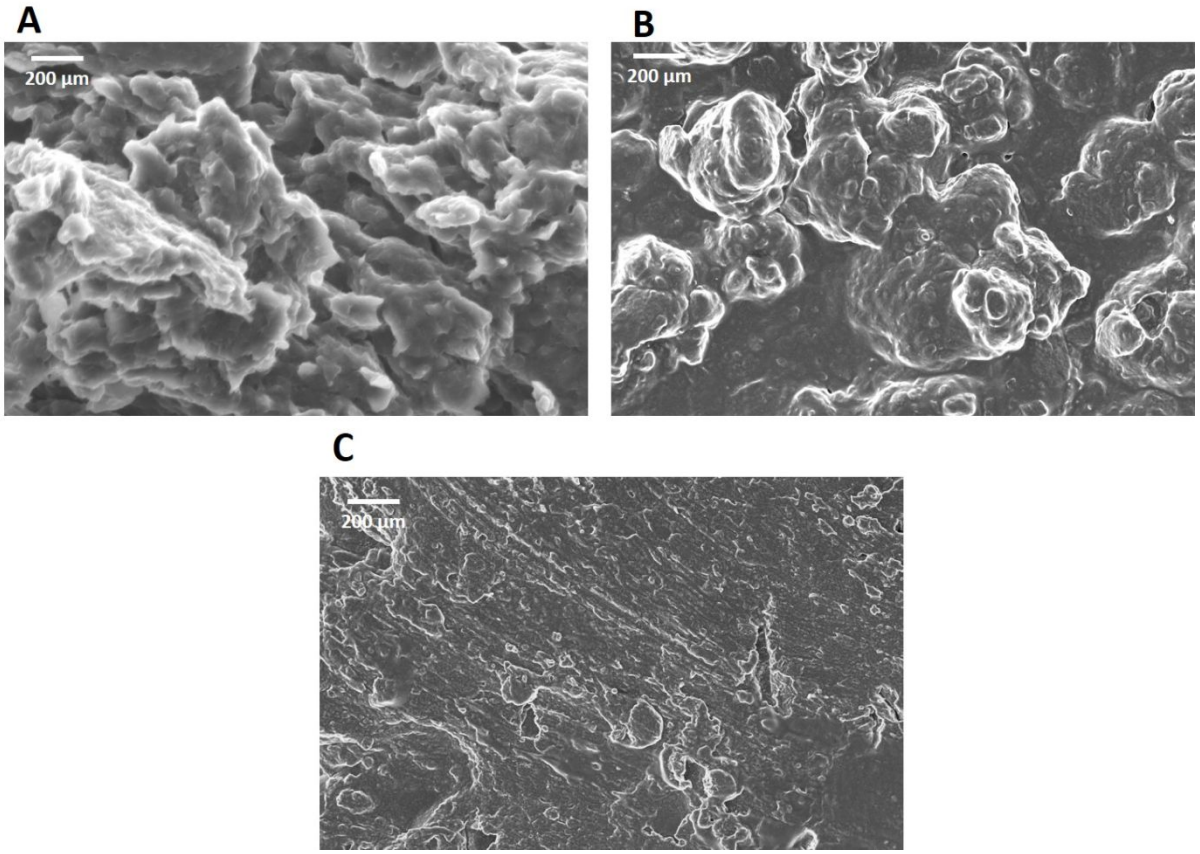


Figure 6. Morphology of hydrogels containing F3 (A), F4 (B) and extract (C) analyzed by SEM.

3.3.6 ***Skin Permeation and Retention Studies.*** The cumulative amount of QU and LU permeating the skin from SPE, F3, and F4 hydrogels is reported in Figure 7. The results showed that the skin permeation of QU and LU in SPE for 24 h was very small at 1.98 ± 0.17% and 1.19 ± 0.12%, respectively. On the other hand, F3 and F4 SLN-based hydrogels permeation increased significantly ($p<0.05$). After applying F3 hydrogel for 24 h, 39.84 ± 3.87% and 37.49 ± 3.09% of the applied QU and LU permeated through the skin, respectively. Meanwhile, QU and LU permeations from hydrogel F4 after 24 hours were calculated to be 21.24 ± 2.09% and 19.24 ± 1.72%, respectively. SLNs have been linked to higher levels of penetration due to their direct effect on skin hydration, causing increased water retention in the stratum corneum as a result of film formation from fatty esters that are bound only by van der Waals interactions to the skin surface. These fatty esters form lipid particles enable the drug to pass through the skin's deep layers.[49,56,65] The skin permeation value of F3 was found to be higher than F4 because of the smaller particle size. In the skin permeation study using a Franz diffusion cell, the rate depends on various factors, such as particle size. Smaller particle sizes could potentially result in more drug permeation. However, there is still ongoing discussion regarding the precise number representing the maximum diameter of nanoparticles that can be penetrated through the skin. Several studies have shown that the smaller the particle size indicates higher and faster skin penetration. Later, Lademann found out that 300–600 nm particles were still able to penetrate the follicle on massage as a consequence of the spacing between the scales on the hair, and suggested that the movement of the hair serves as a directed pump to push the particles into the follicles.[66]

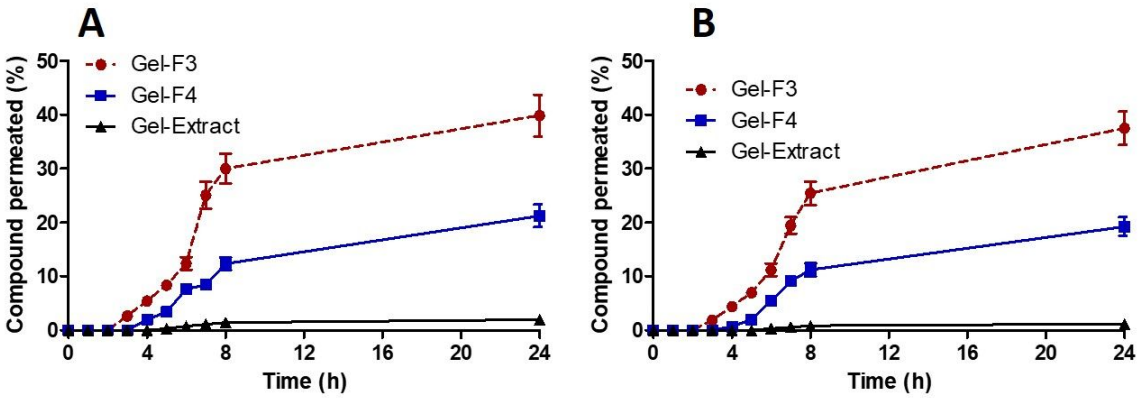


Figure 7. *Ex vivo* permeation profile of QU (A) and LU (B) from Gel-F3, Gel-F4, and Gel-Extract (means ± SD, n = 3).

These data also show good drug retention in the epidermal layer. Since the lipoperoxidation inhibition process occurs mainly when the active component passes through the epidermis, skin drug retention is a significant factor impacting the efficacy of anti-aging therapies. Drug retention increased when SPEs were incorporated into SLNs (Figure 8A-C). Higher SPE lipophilicity and potential effects of SLN on the skin are associated with a 19-fold increase. Regarding drug retention, F4 was greater than F3 due to the bigger particle size.

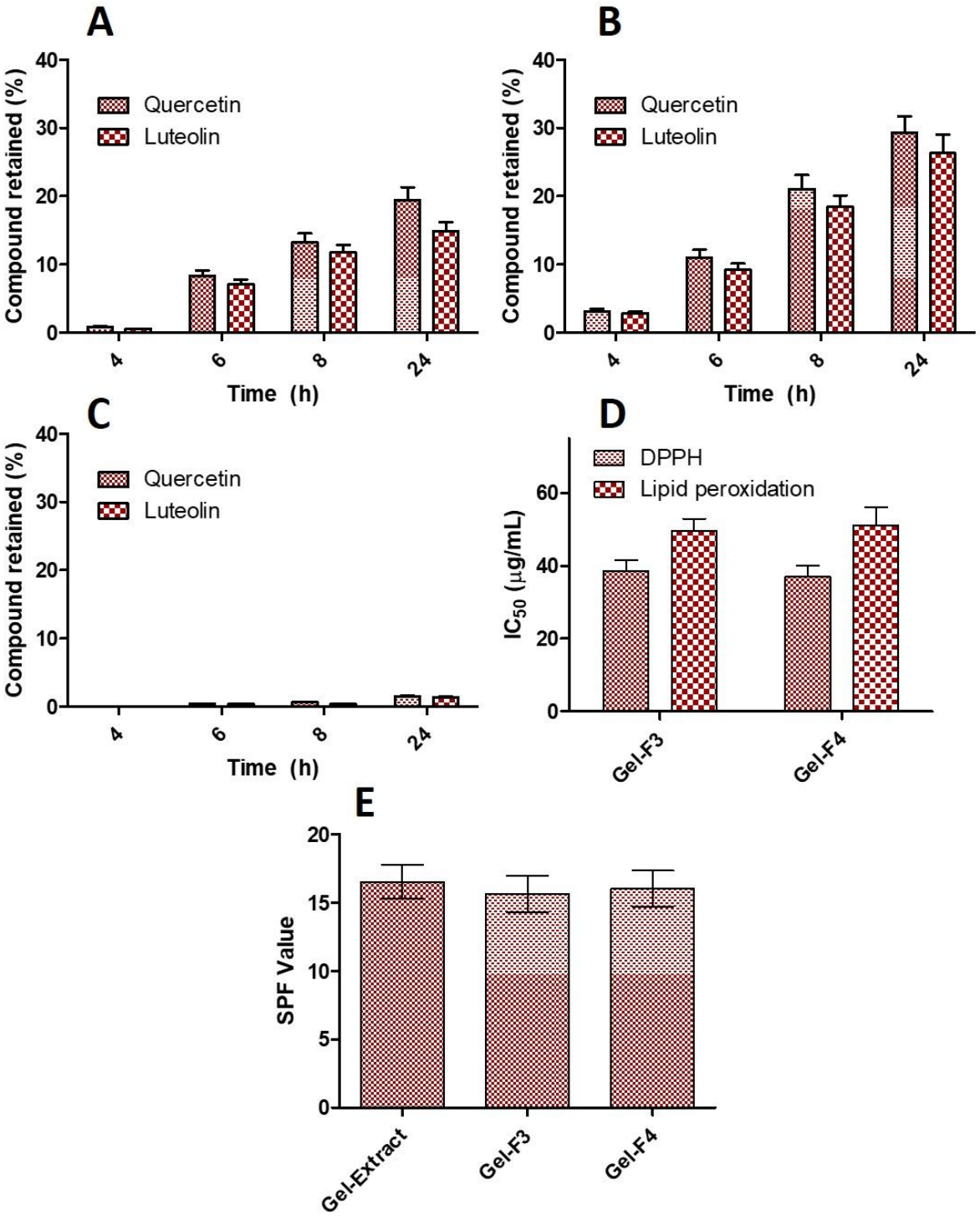


Figure 8. *In vitro* skin drug retention profiles of QU and LU after 24 h permeation study from Gel-F3 (A), Gel-F4 (B), Gel-Extract (C), antioxidant activity (IC50) against DPPH and lipid peroxidation of hydrogel (D), SPF value (E) (means ± SD, n = 3).

The retention profiles of QU and LU can be attributed to the occlusive properties of the previously described SLN, where occlusives can increase skin permeability and hydration. In addition, drug accumulation in the upper skin layer can also occur, resulting in decreased drug flux, and drug residence time in the skin can be extended by creating a reservoir. Thus, these antioxidant agents can provide longer-lasting protection.

3.4 ***Antioxidant Activity and Sun Protective Factor (SPF) of Optimised Formulation.*** The evaluation of antioxidant activity of F3 and F4 hydrogels against DPPH and lipid peroxidation are shown in Figure 8D. IC50 against DPPH was found to be 36.65 ± 2.86 µg/mL and 37.09 ± 3.02 µg/mL for F3 and F4, respectively. Meanwhile, IC50 in inhibition of lipid peroxidation was 49.65 ± 3.32 µg/mL for F3 and 51.23 ± 4.98 µg/mL for F4. Statistical analysis showed that the SPE-loaded SLN formulas F3 and F4 did not differ significantly ($p > 0.05$), so from the results obtained, it can be concluded that the antioxidant effectiveness of SPE was not affected by the formulation produced.

Finally, the formulation was tested for sun protecting factor (SPF) value. SPF is a quantitative measurement carried out to determine the effectiveness of a sunscreen formulation in its ability to protect and prevent skin damage. The *in vitro* SPF values was determined by UV spectrophotometry between the wavelengths of 290-320 nm to verify absorption in the UVB (290–320) regions. Since the UVB being responsible for immediate damage, causing burns, skin cancer. Meanwhile, UVA radiation penetrates deeper and can damage the DNA, oxidize the lipids, and produce dangerous free radicals, which can cause inflammation, break the cellular communication, modify the gene expression in response to stress, and weaken the skin's immune response. But both radiations are linked to skin cancer [69].

The US Food and Drug Administration (FDA) recommends that to help reduce the risk of sun damage, sunscreen products should have an SPF of 15 or higher.[70] In this study, it was found that the SPF value of the hydrogel formulation on SPE and SPE-loaded SLNs, F3 and

F4 hydrogel were 16.54 ± 1.29; 15.69 ± 1.33; 16.03 ± 1.41, respectively (Figure 8E). Based on statistical analysis, the formulations of SPE and SPE-loaded SLNs were not significantly different ($p > 0.05$). These results indicate that SPE incorporation into SLNs does not change SPE's ability to protect skin against UV lights. All formulas have an SPF value> 15 in accordance with the recommendations from the FDA and the results obtained also indicate that this Safflower petal has high photoprotective properties.

Overall, the findings presented in this study indicate that the formulation of SPE-loaded SLNs leads to increased skin penetration and retention of the antioxidant substance of Safflower petals extract (SPE), QU, and LU. Then, after being formulated into the hydrogel, the SLNs also had no haemolytic effect and could adhere to the skin, which helped the user apply the gel and protect the skin against UV radiation. These results have provided new insights into the natural antioxidant compounds contained in safflower petals in developing topical drug delivery systems for these compounds. Moving forwards, future research, including *in vivo* profiles studies, must now be performed to investigate more about the antioxidant activity of SPE after providing this innovative approach to the experimental animals. In addition, it is also necessary to evaluate the safety and risk assessment of SPE-loaded SLNs antioxidant products in order to prevent allergic reactions or significant biological effects when applied the product topically.

4. Conclusion

In this work, the results of the determination of antioxidant compounds of SPE in ethanol extracts as well as their application in the form of SLN-based hydrogels were presented. Quercetin (QU) and luteolin (LU), the hydrophobic substances, were found to be the major chemical constituents isolated from SPE which is known to have antioxidant properties. Furthermore, the inclusion of SPE into SLNs-based hydrogel formulations may favor the QU and LU solubility and penetration as well as its retention on the skin, which is impacting the

efficacy of photoprotective agents. The optimized F3 and F4 hydrogels tested were considered safe due to enhanced skin hydration and no haemolytic effect. Based on the results of antioxidant activity testing and SPF values, SPE-loaded SLNs based hydrogels show values that are in accordance with FDA recommendations and marked antioxidant activity *in vitro* thus displaying enormous potential to be developed as a topical sunscreen to prevent symptoms associated with photo-induced skin aging. Furthermore, it is necessary to conduct *in vivo* method and risk assessment to the appropriate experimental animals to describe this typical antioxidant activity and safety of this innovative approach thereby validating product performance and presenting a major limitation on the extent to which this material responds to the skin.

AUTHOR INFORMATION

Corresponding Author

Correspondence should be addressed to Andi Dian Permana (Faculty of Pharmacy, Hasanuddin University, Indonesia). Email: andi.dian.permana@farmasi.unhas.ac.id

Authors

Nuur Aanisah - Department of Pharmacy, Faculty of Science, Tadulako University, Palu 94118, Indonesia

Sulistiawati - Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia

Yulia Yusrini Djabir - Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia

Rangga Meidianto Asri - Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia

Sumarheni - Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesi

Lutfi Chabib - Department of Pharmacy, Universitas Islam Indonesia, Yogyakarta 55584, Indonesia

Hasyrul Hamzah - Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda, 75124, Indonesia

Author Contributions

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

Notes

The authors declare no competing financial interest.

ACKNOWLEDGEMENT

This work was supported by Penelitian Dasar Kompetitif Nasional, Ministry of Education, Culture, Research and Technology of Republic of Indonesia.

Reference

(1) Young, A. R.; Morgan, K. A.; Harrison, G. I.; Lawrence, K. P.; Petersen, B.; Wulf, H. C.; Philipsen, P. A. A Revised Action Spectrum for Vitamin D Synthesis by Suberythemal UV Radiation Exposure in Humans in Vivo. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118* (40), 1–8. https://doi.org/10.1073/pnas.2015867118.

(2) Morita, A. Current Developments in Phototherapy for Psoriasis. *J. Dermatol.* **2018**, *45* (3), 287–292. https://doi.org/10.1111/1346-8138.14213.

(3) Kohli, I.; Zubair, R.; Lyons, A. B.; Nahhas, A. F.; Braunberger, T. L.; Mokhtari, M.; Ruvolo, E.; Lim, H. W.; Hamzavi, I. H. Impact of Long-Wavelength Ultraviolet A1

and Visible Light on Light-Skinned Individuals. *Photochem. Photobiol.* **2019**, *95* (6), 1285–1287. https://doi.org/10.1111/php.13143.

(4) Lim, H. W.; Kohli, I.; Ruvolo, E.; Kolbe, L.; Hamzavi, I. H. Impact of Visible Light on Skin Health: The Role of Antioxidants and Free Radical Quenchers in Skin Protection. *J. Am. Acad. Dermatol.* **2022**, *86* (3), S27–S37. https://doi.org/10.1016/j.jaad.2021.12.024.

(5) Balupillai, A.; Nagarajan, R. P.; Ramasamy, K.; Govindasamy, K.; Muthusamy, G. *Caffeic Acid Prevents UVB Radiation Induced Photocarcinogenesis through Regulation of PTEN Signaling in Human Dermal Fibroblasts and Mouse Skin*; Elsevier Inc, 2018; Vol. 352. https://doi.org/10.1016/j.taap.2018.05.030.

(6) Fukunaga, A.; Fukushima, S.; Iwata, H.; Nakahara, M.; Sasaki, R.; Baba, N.; Matsunaka, H.; Murakami, Y.; Furue, M.; Nishigori, C. Bioactive Substances in the Stratum Corneum of the Epidermis Found as Indicators of Skin Damage Due to Sun Exposure. *Photodermatol. Photoimmunol. Photomed.* **2022**, *38* (3), 241–249. https://doi.org/10.1111/phpp.12743.

(7) Sample, A.; Zhao, B.; Qiang, L.; He, Y. Y. Adaptor Protein P62 Promotes Skin Tumor Growth and Metastasis and Is Induced by UVA Radiation. *J. Biol. Chem.* **2017**, *292* (36), 14786–14795. https://doi.org/10.1074/jbc.M117.786160.

(8) Plaza, M.; Pozzo, T.; Liu, J.; Gulshan Ara, K. Z.; Turner, C.; Nordberg Karlsson, E. Substituent Effects on in Vitro Antioxidizing Properties, Stability, and Solubility in Flavonoids. *J. Agric. Food Chem.* **2014**, *62* (15), 3321–3333. https://doi.org/10.1021/jf405570u.

(9) Roh, E.; Kim, J. E.; Kwon, J. Y.; Park, J. S.; Bode, A. M.; Dong, Z.; Lee, K. W. Molecular Mechanisms of Green Tea Polyphenols with Protective Effects against Skin

Photoaging. *Crit. Rev. Food Sci. Nutr.* **2017**, *57* (8), 1631–1637. https://doi.org/10.1080/10408398.2014.1003365.

(10) Yang, Q.; Yang, Z. F.; Liu, S. B.; Zhang, X. N.; Hou, Y.; Li, X. Q.; Wu, Y. M.; Wen, A. D.; Zhao, M. G. Neuroprotective Effects of Hydroxysafflor Yellow A against Excitotoxic Neuronal Death Partially through Down-Regulation of NR2B-Containing NMDA Receptors. *Neurochem. Res.* **2010**, *35* (9), 1353–1360. https://doi.org/10.1007/s11064-010-0191-6.

(11) Orgah, J. O.; He, S.; Wang, Y.; Jiang, M.; Wang, Y.; Orgah, E. A.; Duan, Y.; Zhao, B.; Zhang, B.; Han, J.; Zhu, Y. Pharmacological Potential of the Combination of Salvia Miltiorrhiza (Danshen) and Carthamus Tinctorius (Honghua) for Diabetes Mellitus and Its Cardiovascular Complications. *Pharmacol. Res.* **2020**, *153* (June 2019), 104654. https://doi.org/10.1016/j.phrs.2020.104654.

(12) Yu, G.; Luo, Z.; Zhou, Y.; Zhang, L.; Wu, Y.; Ding, L.; Shi, Y. Uncovering the Pharmacological Mechanism of Carthamus Tinctorius L. on Cardiovascular Disease by a Systems Pharmacology Approach. *Biomed. Pharmacother.* **2019**, *117* (June), 109094. https://doi.org/10.1016/j.biopha.2019.109094.

(13) Arpornsuwan, T.; Petvises, S.; Thim-Uam, A.; Boondech, A.; Roytrakul, S. Effects of Carthamus Tinctorius L. Solvent Extracts on Anti-Proliferation of Human Colon Cancer (SW620 Cell Line) via Apoptosis and the Growth Promotion of Lymphocytes. *Songklanakarin J. Sci. Technol.* **2012**, *34* (1), 45–51.

(14) Loo, W. T. Y.; Cheung, M. N. B.; Chow, L. W. C. The Inhibitory Effect of a Herbal Formula Comprising Ginseng and Carthamus Tinctorius on Breast Cancer. *Life Sci.* **2004**, *76* (2), 191–200. https://doi.org/10.1016/j.lfs.2004.06.021.

(15) Wang, M. na; Cao, Y. gang; Wei, Y. xin; Ren, Y. jie; Liu, Y. ling; Chen, X.; He, C.;

Zheng, X. ke; Feng, W. sheng. Saffloflavone, a New Flavonoid from the Flowers of Carthamus Tinctorius L. and Its Cardioprotective Activity. *Nat. Prod. Res.* **2020**, *0* (0), 1–6. https://doi.org/10.1080/14786419.2020.1855167.

(16) Asgarpanah, J.; Kazemivash, N. Phytochemistry, Pharmacology and Medicinal Properties of Carthamus Tinctorius L. *Chin. J. Integr. Med.* **2013**, *19* (2), 153–159. https://doi.org/10.1007/s11655-013-1354-5.

(17) Zhou, Y. Z.; Liu, X. X.; Zheng, X. H.; Zheng, J. Bin. Simultaneous Determination of Quercetin and Luteolin in Dried Flowers by Multivariate HPLC-ECD Calibration. *Chromatographia* **2007**, *66* (7–8), 635–637. https://doi.org/10.1365/s10337-007-0363-y.

(18) Kurkin, V. A.; Kharisova, A. V. Flavonoids of Carthamus Tinctorius Flowers. *Chem. Nat. Compd.* **2014**, *50* (3), 446–448. https://doi.org/10.1007/s10600-014-0983-z.

(19) Censi, R.; Martena, V.; Hoti, E.; Malaj, L.; Di Martino, P. Permeation and Skin Retention of Quercetin from Microemulsions Containing Transcutol® P. *Drug Dev. Ind. Pharm.* **2012**, *38* (9), 1128–1133. https://doi.org/10.3109/03639045.2011.641564.

(20) Kazmi, I.; Al-Abbasi, F. A.; Nadeem, M. S.; Altayb, H. N.; Alshehri, S.; Imam, S. S. Formulation, Optimization and Evaluation of Luteolin-Loaded Topical Nanoparticulate Delivery System for the Skin Cancer. *Pharmaceutics* **2021**, *13* (11), 1–17. https://doi.org/10.3390/pharmaceutics13111749.

(21) Permana, A. D.; Tekko, I. A.; McCarthy, H. O.; Donnelly, R. F. New HPLC–MS Method for Rapid and Simultaneous Quantification of Doxycycline, Diethylcarbamazine and Albendazole Metabolites in Rat Plasma and Organs after Concomitant Oral Administration. *J. Pharm. Biomed. Anal.* **2019**, *170*, 243–253. https://doi.org/10.1016/j.jpba.2019.03.047.

(22) Altamimi, M. A.; Hussain, A.; Alshehri, S.; Imam, S. S.; Alnemer, U. A. Development and Evaluations of Transdermally Delivered Luteolin Loaded Cationic Nanoemulsion: In Vitro and Ex Vivo Evaluations. *Pharmaceutics* **2021**, *13* (8). https://doi.org/10.3390/pharmaceutics13081218.

(23) Han, S. B.; Kwon, S. S.; Jeong, Y. M.; Yu, E. R.; Park, S. N. Physical Characterization and in Vitro Skin Permeation of Solid Lipid Nanoparticles for Transdermal Delivery of Quercetin. *Int. J. Cosmet. Sci.* **2014**, *36* (6), 588–597. https://doi.org/10.1111/ics.12160.

(24) Bose, S.; Du, Y.; Takhistov, P.; Michniak-Kohn, B. Formulation Optimization and Topical Delivery of Quercetin from Solid Lipid Based Nanosystems. *Int. J. Pharm.* **2013**, *441* (1–2), 56–66. https://doi.org/10.1016/j.ijpharm.2012.12.013.

(25) Li, R.; Pu, C.; Sun, Y.; Sun, Q.; Tang, W. Interaction between Soybean Oleosome-Associated Proteins and Phospholipid Bilayer and Its Influence on Environmental Stability of Luteolin-Loaded Liposomes. *Food Hydrocoll.* **2022**, *130* (March), 107721. https://doi.org/10.1016/j.foodhyd.2022.107721.

(26) Guo, C. Y.; Yang, C. F.; Li, Q. L.; Tan, Q.; Xi, Y. W.; Liu, W. N.; Zhai, G. X. Development of a Quercetin-Loaded Nanostructured Lipid Carrier Formulation for Topical Delivery. *Int. J. Pharm.* **2012**, *430* (1–2), 292–298. https://doi.org/10.1016/j.ijpharm.2012.03.042.

(27) Vicentini, F. T. M. C.; Simi, T. R. M.; Del Ciampo, J. O.; Wolga, N. O.; Pitol, D. L.; Iyomasa, M. M.; Bentley, M. V. L. B.; Fonseca, M. J. V. Quercetin in w/o Microemulsion: In Vitro and in Vivo Skin Penetration and Efficacy against UVB-Induced Skin Damages Evaluated in Vivo. *Eur. J. Pharm. Biopharm.* **2008**, *69* (3), 948–957. https://doi.org/10.1016/j.ejpb.2008.01.012.

(28) Sapino, S.; Ugazio, E.; Gastaldi, L.; Miletto, I.; Berlier, G.; Zonari, D.; Oliaro-Bosso, S. Mesoporous Silica as Topical Nanocarriers for Quercetin: Characterization and in Vitro Studies. *Eur. J. Pharm. Biopharm.* **2015**, *89*, 116–125. https://doi.org/10.1016/j.ejpb.2014.11.022.

(29) Chessa, M.; Caddeo, C.; Valenti, D.; Manconi, M.; Sinico, C.; Fadda, A. M. Effect of Penetration Enhancer Containing Vesicles on the Percutaneous Delivery of Quercetin through New Born Pig Skin. *Pharmaceutics* **2011**, *3* (3), 497–509. https://doi.org/10.3390/pharmaceutics3030497.

(30) Netto MPharm, G.; Jose, J. Development, Characterization, and Evaluation of Sunscreen Cream Containing Solid Lipid Nanoparticles of Silymarin. *J. Cosmet. Dermatol.* **2018**, *17* (6), 1073–1083. https://doi.org/10.1111/jocd.12470.

(31) Wissing, S. A.; Müller, R. H. A Novel Sunscreen System Based on Tocopherol Acetate Incorporated into Solid Lipid Nanoparticles. *Int. J. Cosmet. Sci.* **2001**, *23* (4), 233–243. https://doi.org/10.1046/j.1467-2494.2001.00087.x.

(32) Rodrigues, L. R.; Jose, J. Exploring the Photo Protective Potential of Solid Lipid Nanoparticle-Based Sunscreen Cream Containing Aloe Vera. *Environ. Sci. Pollut. Res.* **2020**, *27* (17), 20876–20888. https://doi.org/10.1007/s11356-020-08543-4.

(33) Manea, A. M.; Andronescu, C.; Meghea, A. Green Tea Extract Loaded into Solid Lipid Nanoparticles. *UPB Sci. Bull. Ser. B Chem. Mater. Sci.* **2014**, *76* (2), 125–136.

(34) Khameneh, B.; Halimi, V.; Jaafari, M. R.; Golmohammadzadeh, S. Safranal-Loaded Solid Lipid Nanoparticles: Evaluation of Sunscreen and Moisturizing Potential for Topical Applications. *Iran. J. Basic Med. Sci.* **2015**, *18* (1), 58–63.

(35) Zur Mühlen, A.; Schwarz, C.; Mehnert, W. Solid Lipid Nanoparticles (SLN) for

Controlled Drug Delivery - Drug Release and Release Mechanism. *Eur. J. Pharm. Biopharm.* **1998**, *45* (2), 149–155. https://doi.org/10.1016/S0939-6411(97)00150-1.

(36) Wissing, S. A.; Lippacher, A.; Müller, R. H. Investigations on the Occlusive Properties of Solid Lipid Nanoparticles (SLN). *J. Cosmet. Sci.* **2001**, *52* (5), 313–324.

(37) Khalil, R. M.; Abd-Elbary, A.; Kassem, M. A.; Ghorab, M. M.; Basha, M. Nanostructured Lipid Carriers (NLCs) versus Solid Lipid Nanoparticles (SLNs) for Topical Delivery of Meloxicam. *Pharm. Dev. Technol.* **2014**, *19* (3), 304–314. https://doi.org/10.3109/10837450.2013.778872.

(38) Khalil, R. M.; El-bary, A. A.; Kassem, M. A.; Ghorab, M. M.; Ahmed, M. B. Solid Lipid Nanoparticles for Topical Delivery of Meloxicam: Development and In Vitro Characterization. *Eur. Sci. J.* **2013**, *4* (7), 24–26.

(39) Abdel-Salam, F. S.; Elkheshen, S. A.; Mahmoud, A. A.; Ammar, H. O. Diflucortolone Valerate Loaded Solid Lipid Nanoparticles as a Semisolid Topical Delivery System. *Bull. Fac. Pharmacy, Cairo Univ.* **2016**, *54* (1), 1–7. https://doi.org/10.1016/j.bfopcu.2015.11.002.

(40) Tursilli, R.; Piel, G.; Delattre, L.; Scalia, S. Solid Lipid Microparticles Containing the Sunscreen Agent, Octyl-Dimethylaminobenzoate: Effect of the Vehicle. *Eur. J. Pharm. Biopharm.* **2007**, *66* (3), 483–487. https://doi.org/10.1016/j.ejpb.2007.02.017.

(41) Lu, Y.; Liu, M.; Cao, Y.; Yin, J.; Zhou, H.; Yu, W.; Liu, H.; Wang, J.; Huang, C.; Ma, P.; Que, S.; Gong, C.; Zhao, G. Hydrogel Sunscreen Based on Yeast /Gelatin Demonstrates Excellent UV-Shielding and Skin Protection Performance. *Colloids Surfaces B Biointerfaces* **2021**, *205* (April), 111885. https://doi.org/10.1016/j.colsurfb.2021.111885.

(42) Wang, R.; Wang, X.; Zhan, Y.; Xu, Z.; Xu, Z.; Feng, X.; Li, S.; Xu, H. A Dual Network Hydrogel Sunscreen Based on Poly-γ-Glutamic Acid/Tannic Acid Demonstrates Excellent Anti-UV, Self-Recovery, and Skin-Integration Capacities. *ACS Appl. Mater. Interfaces* **2019**, *11* (41), 37502–37512. https://doi.org/10.1021/acsami.9b14538.

(43) Gwak, M. A.; Hong, B. M.; Park, W. H. Hyaluronic Acid/Tannic Acid Hydrogel Sunscreen with Excellent Anti-UV, Antioxidant, and Cooling Effects. *Int. J. Biol. Macromol.* **2021**, *191* (August), 918–924. https://doi.org/10.1016/j.ijbiomac.2021.09.169.

(44) Tan, F.; Cui, H.; Bai, C.; Qing, C.; Xu, L.; Han, J. Preparation, Optimization, and Transcorneal Permeability Study of Lutein-Loaded Solid Lipid Nanoparticles. *J. Drug Deliv. Sci. Technol.* **2021**, *62* (January), 102362. https://doi.org/10.1016/j.jddst.2021.102362.

(45) Patil, J.; Rajput, R.; Nemade, R.; Naik, J. Preparation and Characterization of Artemether Loaded Solid Lipid Nanoparticles: A 32 Factorial Design Approach. *Mater. Technol.* **2020**, *35* (11–12), 719–726. https://doi.org/10.1080/10667857.2018.1475142.

(46) Priyanka, K.; Sahu, P. L.; Singh, S. Optimization of Processing Parameters for the Development of Ficus Religiosa L. Extract Loaded Solid Lipid Nanoparticles Using Central Composite Design and Evaluation of Antidiabetic Efficacy. *J. Drug Deliv. Sci. Technol.* **2018**, *43*, 94–102. https://doi.org/10.1016/j.jddst.2017.08.006.

(47) Mir, M.; Permana, A. D.; Tekko, I. A.; McCarthy, H. O.; Ahmed, N.; Rehman, A. ur; Donnelly, R. F. Microneedle Liquid Injection System Assisted Delivery of Infection Responsive Nanoparticles: A Promising Approach for Enhanced Site-Specific

Delivery of Carvacrol against Polymicrobial Biofilms-Infected Wounds. *Int. J. Pharm.* **2020**, *587* (July), 119643. https://doi.org/10.1016/j.ijpharm.2020.119643.

(48) Enggi, C. K.; Isa, H. T.; Sulistiawati, S.; Ardika, K. A. R.; Wijaya, S.; Asri, R. M.; Mardikasari, S. A.; Donnelly, R. F.; Permana, A. D. 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.* **2021**, *609* (September), 121182. https://doi.org/10.1016/j.ijpharm.2021.121182.

(49) Khurana, S.; Bedi, P. M. S.; Jain, N. K. Preparation and Evaluation of Solid Lipid Nanoparticles Based Nanogel for Dermal Delivery of Meloxicam. *Chem. Phys. Lipids* **2013**, *175–176*, 65–72. https://doi.org/10.1016/j.chemphyslip.2013.07.010.

(50) Permana, A. D.; Tekko, I. A.; McCrudden, M. T. C.; Anjani, Q. K.; Ramadon, D.; McCarthy, H. O.; Donnelly, R. F. Solid Lipid Nanoparticle-Based Dissolving Microneedles: A Promising Intradermal Lymph Targeting Drug Delivery System with Potential for Enhanced Treatment of Lymphatic Filariasis. *J. Control. Release* **2019**, *316* (September), 34–52. https://doi.org/10.1016/j.jconrel.2019.10.004.

(51) Rostamkalaei, S. S.; Akbari, J.; Saeedi, M.; Morteza-Semnani, K.; Nokhodchi, A. Topical Gel of Metformin Solid Lipid Nanoparticles: A Hopeful Promise as a Dermal Delivery System. *Colloids Surfaces B Biointerfaces* **2019**, *175* (October 2018), 150–157. https://doi.org/10.1016/j.colsurfb.2018.11.072.

(52) Vaghasiya, H.; Kumar, A.; Sawant, K. Development of Solid Lipid Nanoparticles Based Controlled Release System for Topical Delivery of Terbinafine Hydrochloride. *Eur. J. Pharm. Sci.* **2013**, *49* (2), 311–322. https://doi.org/10.1016/j.ejps.2013.03.013.

(53) Zhu, R. R.; Qin, L. L.; Wang, M.; Wu, S. M.; Wang, S. L.; Zhang, R.; Liu, Z. X.; Sun, X. Y.; Yao, S. D. Preparation, Characterization, and Anti-Tumor Property of

Podophyllotoxin-Loaded Solid Lipid Nanoparticles. *Nanotechnology* **2009**, *20* (5). https://doi.org/10.1088/0957-4484/20/5/055702.

(54) Das, S.; Ng, W. K.; Kanaujia, P.; Kim, S.; Tan, R. B. H. Formulation Design, Preparation and Physicochemical Characterizations of Solid Lipid Nanoparticles Containing a Hydrophobic Drug: Effects of Process Variables. *Colloids Surfaces B Biointerfaces* **2011**, *88* (1), 483–489. https://doi.org/10.1016/j.colsurfb.2011.07.036.

(55) De Souza, A. L. R.; Andreani, T.; Nunes, F. M.; Cassimiro, D. L.; De Almeida, A. E.; Ribeiro, C. A.; Sarmento, V. H. V.; Gremião, M. P. D.; Silva, A. M.; Souto, E. B. Loading of Praziquantel in the Crystal Lattice of Solid Lipid Nanoparticles: Studies by DSC and SAXS. *J. Therm. Anal. Calorim.* **2012**, *108* (1), 353–360. https://doi.org/10.1007/s10973-011-1871-4.

(56) Üner, M.; Karaman, E. F.; Aydoğmuş, Z. Solid Lipid Nanoparticles and Nanostructured Lipid Carriers of Loratadine for Topical Application: Physicochemical Stability and Drug Penetration through Rat Skin. *Trop. J. Pharm. Res.* **2014**, *13* (5), 653–660. https://doi.org/10.4314/tjpr.v13i5.1.

(57) Perkin, A. G.; Horsfall, L. H. CXXVI.—Luteolin. Part III. *J. Chem. Soc. Trans.* **1900**, *77* (0), 1314–1324.

(58) Ice, C. H.; Wender, S. H. Quercetin and Its Glycosides in Leaves of Vaccinium Myrtillus. *J. Am. Chem. Soc.* **1953**, *75* (1), 50–52. https://doi.org/10.1021/ja01097a013.

(59) Sadegh Malvajerd, S.; Azadi, A.; Izadi, Z.; Kurd, M.; Dara, T.; Dibaei, M.; Sharif Zadeh, M.; Akbari Javar, H.; Hamidi, M. Brain Delivery of Curcumin Using Solid Lipid Nanoparticles and Nanostructured Lipid Carriers: Preparation, Optimization, and Pharmacokinetic Evaluation. *ACS Chem. Neurosci.* **2019**, *10* (1), 728–739.

https://doi.org/10.1021/acschemneuro.8b00510.

(60) Sharma, M.; Sharma, R.; Jain, D. K.; Saraf, A. Enhancement of Oral Bioavailability of Poorly Water Soluble Carvedilol by Chitosan Nanoparticles: Optimization and Pharmacokinetic Study. *Int. J. Biol. Macromol.* **2019**, *135*, 246–260. https://doi.org/10.1016/j.ijbiomac.2019.05.162.

(61) Bolla, P. K.; Kalhapure, R. S.; Rodriguez, V. A.; Ramos, D. V.; Dahl, A.; Renukuntla, J. Preparation of Solid Lipid Nanoparticles of Furosemide-Silver Complex and Evaluation of Antibacterial Activity. *J. Drug Deliv. Sci. Technol.* **2019**, *49* (August 2018), 6–13. https://doi.org/10.1016/j.jddst.2018.10.035.

(62) Zhou, H. Y.; Zhang, Y. P.; Zhang, W. F.; Chen, X. G. Biocompatibility and Characteristics of Injectable Chitosan-Based Thermosensitive Hydrogel for Drug Delivery. *Carbohydr. Polym.* **2011**, *83* (4), 1643–1651. https://doi.org/10.1016/j.carbpol.2010.10.022.

(63) Ramasamy, T.; Khandasami, U. S.; Ruttala, H.; Shanmugam, S. Development of Solid Lipid Nanoparticles Enriched Hydrogels for Topical Delivery of Anti-Fungal Agent. *Macromol. Res.* **2012**, *20* (7), 682–692. https://doi.org/10.1007/s13233-012-0107-1.

(64) Kelidari, H. R.; Saeedi, M.; Akbari, J.; Morteza-Semnani, K.; Gill, P.; Valizadeh, H.; Nokhodchi, A. Formulation Optimization and in Vitro Skin Penetration of Spironolactone Loaded Solid Lipid Nanoparticles. *Colloids Surfaces B Biointerfaces* **2015**, *128*, 473–479. https://doi.org/10.1016/j.colsurfb.2015.02.046.

(65) Lademann, J.; Richter, H.; Schanzer, S.; Knorr, F.; Meinke, M.; Sterry, W.; Patzelt, A. Penetration and Storage of Particles in Human Skin: Perspectives and Safety Aspects. *Eur. J. Pharm. Biopharm.* **2011**, *77* (3), 465–468. https://doi.org/10.1016/j.ejpb.2010.10.015.

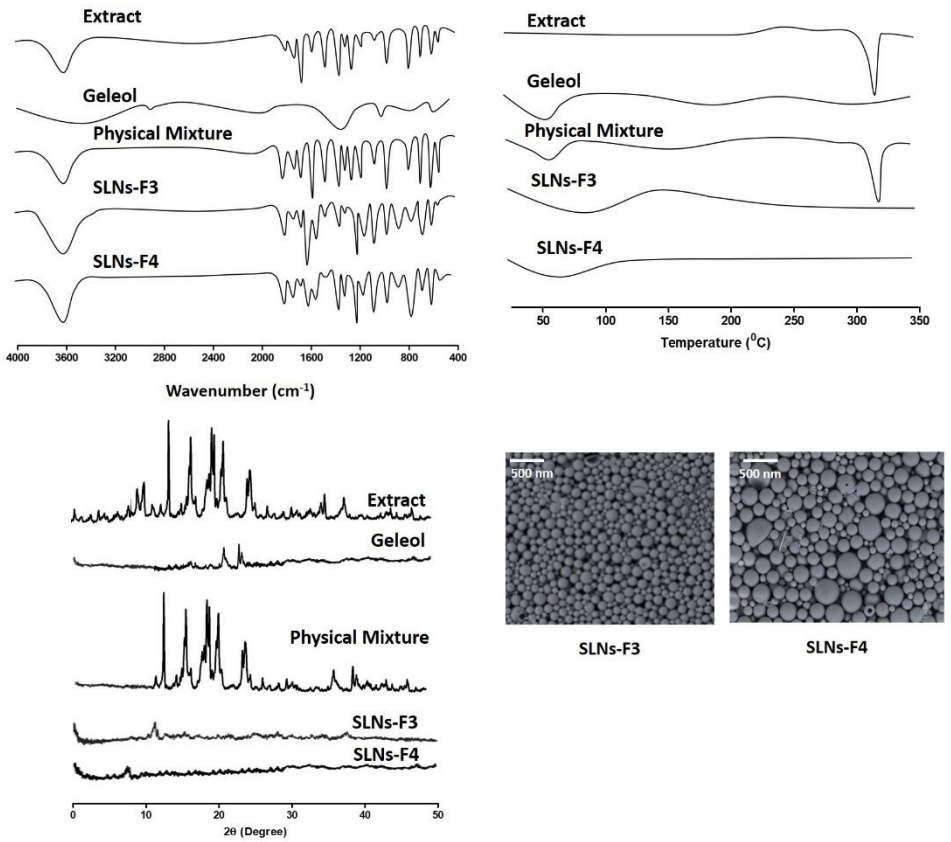
(66) Betageri, G. V.; Deshmukh, D. V.; Gupta, R. B. Oral Sustained-Release Bioadhesive Tablet Formulation of Didanosine. *Drug Dev. Ind. Pharm.* **2001**, *27* (2), 129–136. https://doi.org/10.1081/DDC-100000479.

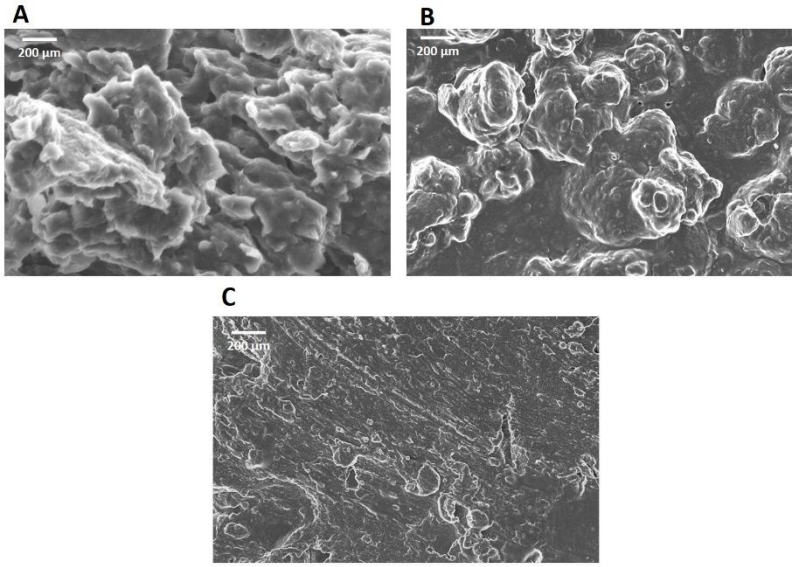
(67) Calixto, G.; Yoshii, A. C.; Rocha E Silva, H.; Stringhetti Ferreira Cury, B.; Chorilli, M. Polyacrylic Acid Polymers Hydrogels Intended to Topical Drug Delivery: Preparation and Characterization. *Pharm. Dev. Technol.* **2015**, *20* (4), 490–496. https://doi.org/10.3109/10837450.2014.882941.

(68) Dupont, E.; Gomez, J.; Bilodeau, D. Beyond UV Radiation: A Skin under Challenge. *Int. J. Cosmet. Sci.* **2013**, *35* (3), 224–232. https://doi.org/10.1111/ics.12036.

(69) FDA. Tips to Stay Safe in the Sun: From Sunscreen to Sunglasses https://www.fda.gov/consumers/consumer-updates/tips-stay-safe-sun-sunscreen-sunglasses (accessed Aug 11, 2022).

884 **TABLE OF CONTENT**





Solid lipid nanoparticles (SLNs) containing Safflower extract

885

886

Journal: Langmuir

Manuscript ID: la-2022-02754m

Title: "Development of solid lipid nanoparticles loaded polymeric hydrogels containing antioxidant and photoprotective bioactive compounds of Safflower (*Carthamus tinctorius* L.) for improved skin delivery"

Response to Reviewers

We thank the reviewer for reviewing the manuscript in detail and providing valuable suggestions for the improvement of our manuscript. We are pleased to submit the revision of the manuscript to Langmuir and addressed all comments, as follows:

Reviewer(s)' Comments to Author:

Reviewer: 1

Recommendation: Major revisions needed as noted.

Comments:

In this manuscript, quercetin and luteolin, the active components of safflower, were extracted and then encapsulated in a hydrogel based on solid lipid nanoparticles to enhance solubility and skin permeability. It has been proved that this formulation has good antioxidant activity and sunscreen effect. However, there are major problems in the writing of the manuscript, which makes it difficult for readers to read. At the same time, it lacks professional expression and makes mistakes on many common-sense issues. In addition, the design of the material system lacks highlights, and the experimental scheme needs to be improved. The problems in the manuscript are as follows:

Response: We thank the reviewers for reviewing the manuscript in detail and providing valuable suggestions for the improvement of our manuscript. All mistakes made have been addressed in detail, as follows:

1. The abbreviation SPE appears for the first time in the abstract, but its full name is not stated. Meanwhile, the keyword photoprotective does not appear in the abstract.

   Response:

   We thank the reviewer for the comment. We have added the complete name for SPE in the abstract and included the keyword photoprotective in it.

2. In the manuscript, line 158, FeCl2 is miswritten and line 171, the reversed-phase column size (150 × 4.6 mm, 5 m) should be changed to (150 × 4.6 mm, 5 µm). In line 225, the heating rate of 10 C/min should be changed to 10℃/min. In line 329, the gravity acceleration (980 m/s2) should be changed into 9.8 m/s2. In line 517, SLNs is incorrectly expressed. In table4, the number 0,97 should be changed into 0.97.

   Response:

   We thank the reviewer for pointing this out. We have corrected those misspelling in our revised manuscript.

3. In the manuscript, italics should be used in vivo and in vitro. In addition, the use of many units in the manuscript should also be standardized, such as µL and mL.

   Response:

   We thank the reviewer for pointing this out. We have corrected those misspelling in our revised manuscript.

4. There are many figures in the manuscript that are not clear enough, as shown in Figure 6, and it is recommended to redraw. In addition, some column charts have uneven color filling, as shown in Figure 1B, and it is recommended to redraw.

   Response:

   We thank the reviewer for pointing this out. As the reviewer suggestion, we have redrawn the unclear figures.

5. The SEM images (Figure 4D) of SLNs formulations lack scale, which is very important. In addition, the author developed a hydrogel formulation based on solid lipid nanoparticles (SLNS), but did not conduct any characterization of the hydrogel morphology, which is not rigorous.

   Response:

   We thank the reviewer for pointing this out. We have added the scale in the SEM images (Figure 2D). Moreover, we have conducted the SEM analysis to observe the morphology of SLNs based hydrogel. Following the observation using SEM, it was found that all hydrogel formulations possessed rough surface with higher brawl borders. The SEM images of all formulations are exhibited in Figure 6. As shown, the SLNs were completely entrapped in the hydrogel matrices. Therefore, this could potentially provide sustained release behaviour of the bioactive compounds from the Carbopol-based hydrogel formulations.(Dudhipala and Gorre, 2020)

6. In the in vitro penetration experiment of gel (as shown in FIG. 6 and FIG. 8), the time interval of measurement points was set unreasonable. Follow-up measurements should be taken during periods of rapid increase in data.

   Response:

   We thank the reviewers for the comments. However, in general, the *in vitro* release profiles and the *ex vivo* permeation studies from numerous compounds, including synthetic and natural compounds have been widely performed using the time interval that we performed in this study (Alhidayah *et al.*, 2021; Ananda *et al.*, 2021; Anjani *et al.*, 2022; Donnelly *et al.*, 2013; Enggi *et al.*, 2021; Permana *et al.*, 2019, 2020; Zhang *et al.*, 2011). Some studies have shown that the sustained release profile of compounds from several delivery system can be observed in 24 hours. Importantly, as the hydrogel formulation developed in our study was intended for daily application, we performed 24 h with several time intervals to investigate the *in vitro* release and the *ex vivo* permeation behaviours.

7. What is the maximum diameter of nanoparticles that can be penetrated through the skin and whether relevant experiments have been done to ensure that the formulation can meet the conditions of skin penetration?

   Response:

   We thank the reviewer for the question. In this study, we developed SPE-loaded SLNs to improve skin penetration. SLNs have been linked to higher levels of penetration due to their direct effect on skin hydration, causing increased water retention in the stratum corneum as a result of film formation from fatty esters that are bound only by van der Waals interactions to the skin surface. These fatty esters form lipid particles enable the drug to pass through the skin's deep layers (dermis and beyond). However, there is still ongoing discussion regarding the precise number representing the maximum diameter of nanoparticles that can be penetrated through the skin. Several studies have shown that the smaller the particle size indicates higher and faster skin penetration. Later, Lademann found out that 300–600 nm particles were still able to penetrate the follicle on massage as a consequence of the spacing between the scales on the hair, and suggested that the movement of the hair serves as a directed pump to push the particles into the follicles (Lademann *et al.*, 2011). We have added this explanation in the revised manuscript.

8. The study from the manuscript found that the drug-loaded solid lipid nanoparticle hydrogels showed advantages in many aspects, but achieved almost the same effect in the sunscreen test as the hydrogels directly loaded with drugs. Is it contradictory? What is the significance of this designed drug delivery gel formulation based on solid lipid nanoparticles?

   Response:

   In this study, we develop solid lipid nanoparticles (SLNs)-based hydrogel formulations to enhance the solubility and penetration of two bioactive compounds found in safflower petal extract (SPE) to act as skin protection agents from the UV lights. Based on statistical analysis, the formulations of SPE and SPE-loaded SLNs were not significantly different ($p > 0.05$). These results indicate that SPE incorporation into SLNs did not change SPE's ability to protect skin against UV lights. However, it makes the extract significantly increase its solubility, penetration and retention to the skin.

9. Safety is a necessary issue to be considered for cosmetics. Whether the formulation designed by the author will cause allergies, and whether the product will interfere with hormones and reproductive system after penetrating into the skin and entering the human body are all questions to be considered. However, there is no description of these issues in the manuscript.

   Response: We thank the reviewer for pointing this out. Indeed, we agreed that it is necessary to evaluate the safety and risk assessment of SPE-loaded SLNs antioxidant products in order to prevent further allergic reactions or significant biological effects when applied topically. Thus, we mentioned this issue at the end of our discussion which suggested for future research.

Response to Reviewers

We thank the reviewer for reviewing the manuscript in detail and providing valuable suggestions for the improvement of our manuscript. We are pleased to submit the revision of the manuscript to Langmuir and addressed all comments, as follows:

Reviewer(s)' Comments to Author:

Reviewer: 2

Recommendation: Other

Comments:

The manuscript describes the physicochemical characterization and biological activity studies on some solid lipid nanoparticles. The works are trustworthy and appears to be systematically carried out. However, the manuscript suffers from several lacunae. It needs thorough revision before a final decision be made. The major concerns are the length, number of figures/images and proper interpretation of the experimental results.

Some specific comments:

1. There are several wrongly constructed sentences. Sometimes the statements are self-contradictory.

   Response:

   We thank the reviewer for the comment. Following the reviewer suggestion, we have checked the manuscript thoroughly and rearranged it carefully.

2. Line 30: In the abstract what do the authors mean by the term “drug”?

   Response:

   We thank the reviewer for the comment. The term “drug” refers to the bioactive compounds found in safflower petal extract (SPE), namely quercetin (QU) and luteolin (LU). We have corrected this in the revised manuscript.

3. Line 29-32: It is mentioned that the drug is trapped in the lipid matrix. If it is so, then how could the drug release be faster? And in any drug delivery system, the prerequisite is the process of sustained release.

   Response:

   We thank the reviewer for pointing this out. Combining SLNs and hydrogels will display both component advantages such as the protection and the improvement of

the solubility of the lipophilic active molecule, while improving the drug release. The faster release could be due to the immediate release of the compounds from the surface of lipid core (Permana *et al.*, 2019). Even though the drug release from SPE-loaded SLN-bearing hydrogel formulations was faster, the sustained drug release for 24 hours was still be observed, suggesting their applicability as the carrier to protect the skin from the UV-rays all day.

4. In the abstract the acronyms like “SPE” and “SPF” were used. It is preferred that their full forms would be used in their first appearances. In fact, throughout the entire manuscript, many acronyms were used without their introduction.

   Response:

   We thank the reviewer for the comment. We have re-read the manuscript thoroughly and completed word/abbreviation similar throughout the text.

5. In the abstract section itself I would expect some comments on the biocompatibility of the formulations. Are they cytotoxic? In fact, authors have carried out the same, however, they remain silent about the biocompatibility, which is considered as one of the important markers of any drug delivery system.

   Response:

   We thank the reviewer for the suggestion. As a result of the reviewer comment, we have provided information about the biocompatibility results in the abstract which have been done through in vitro hemolytic assay. We believe that the revised abstract has reflected the content of the manuscript.

6. The introduction section seems to be quite lengthy and unorganized. It should be more focused and specific. How the present set of works are going to shed new lights over the existing lacunae? What would be the new insights? Authors may mention them at the end of the introduction section.

   Response:

   We thank the reviewers for the suggestions. Following the reviewer's suggestion, we have rearranged the introduction section from 844 words to 760 words and we believe it has become more focused and specific. We have also added a sentence regarding the research gap to gain the new insight in the field.

7. In the introduction section a brief description on “Geleol” would be more impressive for general readers.

Response: We thank the reviewer for the suggestion. Following the reviewer suggestion, we have provided the required information, as follows:

"Several types of solid lipids have been used to prepare SLNs (Abdel-Salam *et al.*, 2016; Khalil *et al.*, 2013, 2014) but Geleol® (glycerol monostearate) is a fascinating since even at low concentrations is enough to produce SLNs that, after cooling, have a semisolid appearance.(Abdel-Salam *et al.*, 2016; Khalil *et al.*, 2013) This occurred due to the lower melting point and composition of Geleol® which contains high amount of monoglycerides, that can form hydrogen bonds with water molecules to promote swelling and exhibit a semisolid appearance.(Abdel-Salam *et al.*, 2016)"

8. In the beginning of the experimental section, I would expect the details on the chemicals used, (purity, make and country of origin, etc.) and the instruments.

   Response:

   We thank the reviewer for the comment. Following the reviewer suggestion, we have included the information in the revised manuscript.

9. What is the rationale behind preparing sixteen different formulations (Table 2)?

   Response:

   We thank the reviewer for the question. SLNs are composed of high melting point lipids as a solid core coated by surfactant. Surfactants reduce the interfacial tension between the hydrophobic surface of the lipid core and the aqueous environment and, therefore, stabilize the SLNs structure. The effects of various parameters were then studied on the physicochemical properties of obtained SPE-loaded SLNs, such as difference in Geleol® concentration, duration of homogenization, and type of surfactants (Tween 80 and PVA (Mw=9-10 kDa)). Therefore, following the reviewer comment, we have added the discussion in section 3.1 SLNs formulation about it.

   The effects of various parameters were studied in optimizing SPE-loaded SLNs formulation, such as difference in Geleol® concentration, duration of homogenization, and type of surfactants (Tween 80 and PVA) used. Based on previous research, Geleol® was selected because it has the lowest melting point of ~55 °C. This could lead to the lowest viscosity in the medium, resulting in increased homogenization and sonication efficiency to produce smaller particles.(Permana *et al.*, 2019) Additionally, it showed

a semisolid appearance after cooling, even in low concentrations.(Abdel-Salam *et al.*, 2016) In the screening of surfactants, Tween 80 and PVA were used because of their non-ionic nature.(Abdel-Salam *et al.*, 2016; Rostamkalaei *et al.*, 2019)

10. Usually, active ingredients from plant products are extracted from the dried and pulverized plant components. What was the rationale behind extracting from the raw plants? Also, I would expect a line of statement as why different proportions of water-alcohol mixtures were used in the extraction procedure.

    Response:

    We thank the reviewer for the comment. In this study, we use the dried and pulverized Safflower petals for the extraction. We have specifically mentioned it in the supplementary method section.

    Then, we use different proportions of water-alcohol mixtures based on literature studies. A mixture of ethanol and water was used because both solvents could extract different types of bioactive compounds. Two bioactive compounds in Safflowers namely quercetin and luteolin are antioxidant compounds of the flavonoid class which are generally extracted using water-alcohol mixtures. So that the variation of solvent will affect the extraction yield as well as the antioxidant activity. In fact, our results show that the bioactive compounds in SPE are more soluble in 100% ethanol so that they show maximum DPPH radical scavenging activity.

11. Equation 1: While calculating the yield of extraction, should one take into account the whole flower? In the denominator, should it be Wsafflower or Wsafflower petals?

    Response:

    We thank the reviewer for pointing this out. We have made it clear in the Equation 1 that we used the Wsafflower petals for calculating the yield of extraction

12. The experimental part is also quite lengthy. It needs to be substantially shortened.

    Response:

    We thank the reviewer for the suggestion. Following the reviewer suggestion, we have shortened the experimental section.

13. Table 2: Which Tween was used as stabilizer? What is the molecular weight of PVA?

    Response:

Thank you for the comment. We have added the full name of Tween 80 and molecular weight of PVA in the revised manuscript

14. 2.2.3.1. Particle Size, PDI, and Zeta Potential studies: What was the scattering angle? What is the model number of DLS? What type of laser was used?

    Response:

    We thank the reviewer for the question. The assessment of mean particle size, polydispersity index (PDI), and zeta potential of the SPE-loaded SLNs was conducted by dynamic light scattering at 90° scattering angel using using a Malvern Zeta Sizer® (Malvern Instruments Ltd, UK), at 25 °C. Specifically, Z-average (d.nm) with intensity (%) was used to represent the particle size. We have included the information in the revised manuscript.

15. 2.2.3.4. DSC studies: The scan rate seems to be quite high. Please cite proper references where such high scan rates were used.

    Response: We thank the reviewer for the comment. Several studies regarding the thermal analysis of SLNs used variations of the scanning rate from 1-50 ml/min under nitrogen purge (Das *et al.*, 2011; De Souza *et al.*, 2012; Zhu *et al.*, 2009). This scan rate level will give different transition temperatures and peak shapes on the DSC melting curve. At high scanning rates, there is no time for the heat to be transmitted from the heating elements of the DSC cell to the sample, resulting in a higher peak transition. Moreover, too high heating rates will give the smooth shape of the melting curve and, as a result, detailed information may get lost. Meanwhile, at a lower scanning rate it also causes a narrowing of the melting endotherms or crystallization exotherms. A low scan rate (1 °C/min) gives several peaks that are close together. Thus, in the current study we chose to use 10 °C/min to produce a distinguishable thermal transition (Üner *et al.*, 2014).

16. 2.2.3.6. Scanning Electron Microscope: Please provide some details on the sample preparation. What was the coating agent? What was the applied voltage for SEM analyses?

    Response:

    We thank the reviewer for the question. Morphological examination of SPE-loaded SLN was examined using a scanning electron microscope (SEM) (JEM-1400Plus, Tokyo,

Japan). Initially, 100 µL of SLNs were air-dried and coated with gold under vacuum sputter. The analysis was carried out at 15 kV.

17. 2.2.3.7. Solubility analysis. The saturated solubility analysis of QU and LU: What are AU and LU?

    Response:

    We thank the reviewer for pointing this out. QU and LU are referred to quercetin and luteolin, respectively.

18. Authors should clearly mention the ethical guidelines regarding the biological studies involving animals.

    Response:

    We thank the reviewer for pointing this out. We have included this in the revised manuscript.

19. Please mention the positive and negative controls while performing the biological activity tests (biocompatibility and skin permeation tests).

    Response:

    We thank the reviewer for pointing this out. We have added the positive and negative controls in the biological activity tests.

20. Sections 3.1-3.4: These sections are not so relevant, and can be moved to the supplementary section.

    Response:

    We thank the reviewer for the suggestion. Following the reviewer suggestion, we have moved the Sections 3.1-3.4 to the supplementary section.

21. It is not unexpected that "The maximum DPPH radical scavenging activity was produced by the extract made with 100% ethanol (Figure 1E)", and in fact this was also my concern regarding the extraction of SPE using different blends of ethanol +water.

    Response:

    We thank the reviewer for pointing this out. In this study we used different blends of ethanol + water as extraction media based on references from several journals which mostly used these media (Bacchetti *et al.*, 2020; Karimkhani *et al.*, 2016; Kilic Buyukkurt *et al.*, 2021; Kurkin and Kharisova, 2014; Zhang *et al.*, 2020). In fact, our

results show that the bioactive compounds in SPE are more soluble in 100% ethanol so that they show maximum DPPH radical scavenging activity.

22. Figure 3A: Authors should also mention that higher the amount of Tween, smaller were the sizes for the SLNs. Besides, presence of larger quantities of Tweens lead to lower values of PDI.

Response:

We thank the reviewer for the suggestion. Following the reviewer suggestion, we have provided the required information, as follows:

"The particle size of SPE-loaded SLNs prepared with higher concentrations of Tween 80 were seemed beneficial to slightly reduce the particle size. Besides, it also led to lower the PDI values."

23. Authors should comment on the stability of the SLN formulations. Have they tried to monitor the size as function of time? Shelf-life is an important marker of drug delivery systems.

Response:

We thank the reviewer for the suggestion. We have added the additional data regarding the stability study in terms of particle size.

In an attempt to assess the stability of the SLNs, the particle size was observed for 1 month at 25°C. The result of this study is depicted in Figure 3, indicating that despite the increase in the particle size, there was no significant difference ($p < 0.05$) after 1 month storage. Accordingly, it could be concluded that both formulations were physically stable.

24. 3.6.2 FTIR Study and 3.6.4 PXRD Study: I would expect the description on the FTIR/XRD studies of the excipients (precursors) and physical mixtures and subsequent comparison with the different formulations. Location of the drugs in the SLN matrix could also be explained by suitably analyzing the FTIR data.

Response:

We thank the reviewer for the comment. We have performed the FTIR, XRD and DSC analyses of the excipient and the physical mixtures. We have also discussed the results accordingly in the revised manuscript. However, as explained in our manuscript, we

selected F3 and F4 as the optimum formulation for the further studies. Accordingly, we only analyzed these two formulations.

Later, we have mentioned about the location of the drugs in the SLN matrix according to the FTIR data.

"This indicates an adequate entrapment of QU and LU in a lipid matrix and no chemical interaction between the drug and excipients used in the formulation."

25. 3.6.3 DSC analysis: interpretation of the DSC data are wrong. Authors should note that the SLNs show characteristic transition peaks in the range of 30-80 deg C. And in fact, it is clear from Figure 4B that there are some transitions taking place. Authors should narrow down their studies within this temperature range.

    Response:

    We thank the reviewer for pointing this out. Following the comment, we have added the explanation regarding the transition peaks in the range of 30-80 deg C in our revised manuscript:

    "The nanoparticles show glass transition characteristics that occurred between 30-80 °C, as compared to the extract thermogram. The appearance of the glass transition and the decrease in melting temperature might be attributed to the reduced particle size and increased surface area (Gibbs–Thompson effect)."

26. Scale bars/magnification in the SEM images are missing. Authors should also try to compare the DLS and SEM data.

    Response:

    We thank the reviewer for pointing this out. We have added the scale bars to the SEM images and also compared the result to the DLS data

27. Line 489: Particles were not "spherical vesicles". Vesicles can not be visualized by SEM. I understand that the authors are not dealing with the vesicles.

    Response:

    We thank the reviewer for pointing this out. We have deleted the word "vesicles" in our revised manuscript.

28. Table 4: I would expect the presentation of regression coefficient values. This would eventually enable readers to understand the superiority of a particular model.

    Response:

    We thank the reviewer for pointing this out. The values shown in Table 4 are the values of the regression coefficient to determine the best-fit model for SPE-loaded SLNs release.

29. Figure 6: In the Y axes of each graph, should it be “%Cumulative release”?

    Response:

    We thank the reviewer for pointing this out. Needful is done.

Response to Reviewers

We thank the reviewer for reviewing the manuscript in detail and providing valuable suggestions for the improvement of our manuscript. We are pleased to submit the revision of the manuscript to Langmuir and addressed all comments, as follows:

Reviewer(s)' Comments to Author:

Reviewer: 3

Recommendation: Major revisions needed as noted

Comments:

Overall, the paper has merit. The work is detailed, thorough and sound research. As the article is written, it is unclear as to what the novelty of the work is. In addition, there are a number of items with the paper that should be addressed before publication:

Thank you for your great comments. The novelty of the work is to determine which solvent produce maximum bioactive components of safflower while maintaining antioxidant activity. Then it is encapsulated in a hydrogel based on solid lipid nanoparticles to increase solubility and skin permeability so that it can be an alternative for skin protection against UV radiation. Following the comments, we have addressed all comments thus we believe that our revised manuscript has greatly improved.

1. Line 51: shouldn't the word "cancer" come after the words "third most common"

   Response:

   We thank the reviewer for the suggestion. Following the reviewer suggestion, we have modified the sentence in the revised manuscript.

2. Line 80-82: the authors state that various approaches and carrier systems have been studied, but yet no references of the types of approaches or carrier systems are provided. In addition, the introduction in general does not provide an adequate overview of the different carrier systems developed to date and how their system is an innovation in the technology.

   Response:

We thank the reviewer for pointing this out. We have added the appropriate references for these statements. The references use different carrier systems for quercetin and luteolin for application to the skin such as microemulsion, liposomes, nanostructured lipid carriers (NLCs), and solid lipid nanoparticles (SLNs). However, SLNs seem to be the main determinant of the extent of lipophilic compounds to penetrate into the epidermal layer of the skin after topical application, resulting in increased bioavailability and greater efficacy.

3. Line 265: The 1 h incubation time seems short, why was this short exposure time chosen.

   Response:

   We thank the reviewer for the question. The established method for in vitro hemolytic assay is incubation for 1 h at 37 °C. We have cited the reference in our manuscript (Enggi *et al.*, 2021; Mir *et al.*, 2020).

4. Line 336-338: Why was only 290-320 nm wavelengths selected as opposed to the full range of UVA&B wavelengths (280-400 nm)?

   Response:

   We thank the reviewer for the question. In this study, the scan was carried out between the wavelength of 290-320 nm to verify absorption in the UVB (290–320) regions. Since the UVB being responsible for immediate damage, causing burns, skin cancer. Meanwhile, UVA radiation penetrates deeper and can damage the DNA, oxidize the lipids, and produce dangerous free radicals, which can cause inflammation, break the cellular communication, modify the gene expression in response to stress, and weaken the skin's immune response. But both radiations are linked to skin cancer

5. Line 364-365: The authors state that the data shows "significant increase in TPC and TFC compared to the TPC and TFC achieved with E100, E50, E25 and WE." However, it is unclear where the significance lies, i.e. is it between all ethanol solutions and water or between each ethanol solution and water, etc. Clarification should be provided.

   Response:

   We thank the reviewers for the comment. The significant increase in TPC and TFC was observed with E100 compared to the TPC and TFC achieved with E75, E50, E25 and WE.

6. Line 368: Figure 1. Any significant differences between the bars should be labeled on the graphs, i.e. which data is considered to be significantly different from other data?

   Response:

   We thank the reviewer for the question. We have added the significance label on the figures.

7. Line 405: Figure 2: Significance needs to be identified for the data in graph B. Labeling of some of the other key peaks in the chromatogram (Figure 2A) would be helpful.

   Response:

   We thank the reviewer for the question. We have added the significance label of the key peaks in the chromatogram.

8. Line 433-434: The authors state that the higher concentration of Geleol in SLNs increased particle size and PDI of SLNs as evidenced by graphs 3A & B. In general, this does not appear to be true except for the PVA surfactant case. The Tween 80 surfactant seemed to produce a more consistent particle size and PDI regardless of Geleol concentration. Also, no indication on any significant differences between the data presented in Graphs for Figure 3 A-D and graph D is not labeled.

   Response:

   We thank the reviewer for pointing this out. Indeed, we are agreed with the reviewer comment, thus we add those statement to the revised manuscript. Also, we have added the significance differences on the figures.

9. Lines 439-443: What is the implication of the fact that their SLNs had zeta potentials in the narrow range of -29.33 and -33.91 mV?

   Response:

   We thank the reviewer for the comment. Zeta potential is one of the physical stability parameters of nanoparticle dispersion. The narrow range of zeta potentials due to the use of same lipid types with different concentrations. All formulations exhibited a negative charge attributed to the anionic nature of the lipids.

10. Lines 444-447: This statement appears to be correct for the F1-F4 formulations, but not necessarily for the F9-F12 formulations. Please clarify.

    Response:

    We thank the reviewer for the comment. F1-F4 and F9-12 use Tween 80 with different concentrations. F1-F4 uses Tween 80 and F5-F8 uses PVA as a stabilizer, it shows a

decrease in particle size and increased encapsulation efficiency (EE) of QU and LU for F1-F4. Likewise, with the F9-12 when compared to the F13-F16.

11. Lines 504-507: Are the aqueous solubility values significantly different from one another. They appear to be when comparing QU & LU SPEs to SLNs F3 & F4 values, but was a test of significance performed? The n-octanol solubility does not appear to be significant different but a test would need to be performed to verify.

    Response:

    We thank the reviewer for the comment. The increase in aqueous solubility of the QU & LU SPEs to SLNs F3 & F4 values were significantly different ($p < 0.05$) but the increase in solubility in n-octanol was not significantly different due to the hydrophobic nature of the compound.

12. Line 508: The authors state "This significant increase was due to..." What significant increase? The aqueous solubility or n-octanol? Since it is unclear whether a test of significance was performed, how can it be stated that it was a significant increase?

    Response:

    We thank the reviewer for the question. What we mean is a significant increase in aqueous solubility. The statistical significance is defined as a p-value is less than 0.05

13. Lines 526-527: The word "respectively" needs to added immediately after "F3" and "F4". Also, for the in vitro drug release, were the release values significantly different between the SPE and respective SLNs?

    Response:

    We thank the reviewer for the comment. Needful is done. When SPE was incorporated into SLNs, the release of QU and LU became significantly faster ($p < 0.05$), reaching 84.98 ± 7.43% and 76.81 ± 6.43% for F3, respectively, and 73.14 ± 6.05% and 66.34 ± 5.38% for F4, respectively

14. Figure 6: The y-axis scale are different for graphs A/B compared to C. It may be more helpful to use the same y-axis scale to better illustrate the difference of the drug release amount. It may be better to show 2 graphs - one for QU and one for LU for all 3 carrier platforms, i.e F3, F4 and SPE, as opposed to showing 3 graphs for each carrier platform and QU and LU on the same plot.

    Response:

We thank the reviewer for the suggestion. Following the reviewer suggestion, we have re-drawn the figure.

15. Line 548: The word "respectively" should be added after LU.

    Response:

    We thank the reviewer for pointing this out. Needful is done.

16. Line 554: The word "respectively" should be added after F4.

    Response:

    We thank the reviewer for pointing this out. Needful is done.

17. Line 572: The word "respectively" should be added after 4.87%.

    Response:

    We thank the reviewer for pointing this out. Needful is done.

18. Line 582: "($p<0.05$)" should be added after the word "significantly".

    Response:

    We thank the reviewer for the suggestion. Following the reviewer suggestion, we have added the word in the revised manuscript.

19. Line 586: The word "respectively" should be added after 1.72%.

    Response:

    We thank the reviewer for pointing this out. Needful is done.

20. Figure 8: again the data may be better presented using 2 graphs instead of 3 as mentioned in item 14 (Figure 6) above.

    Response:

    We thank the reviewer for the suggestion. Following the reviewer suggestion, we have re-drawn the figure.

21. Figure 9: Using similar y-axis scale for graphs A-C would be helpful and more clearly illustrate the differences in the data.

    Response:

    We thank the reviewer for the suggestion. Following the reviewer suggestion, we have re-drawn the figure.

22. Line 614: The authors refer to figure 7E for the bioadhesive strength of each hydrogel, yet graph 7E is SPE data. Please clarify. Also, from the SPF data, it looks like the team did not achieve their goal in the research of trying to demonstrate greater protection with the SLNs. This is not properly discussed in the discussion section.

Response:

We thank the reviewer for the comment. Figure 7E refers to the bioadhesive strength of each hydrogel formulation in comparison with blank hydrogel.

In this study, it was found that the SPF value of the hydrogel formulation on SPE and SPE-loaded SLNs, F3 and F4 hydrogel were 16.54 ± 1.29; 15.69 ± 1.33; 16.03 ± 1.41, respectively. Based on statistical analysis, the formulations of SPE and SPE-loaded SLNs were not significantly different ($p > 0.05$). These results indicate that SPE incorporation into SLNs does not change SPE's ability to protect skin against UV lights.

References:

Abdel-Salam, F.S., Elkheshen, S.A., Mahmoud, A.A. and Ammar, H.O. (2016), "Diflucortolone valerate loaded solid lipid nanoparticles as a semisolid topical delivery system", *Bulletin of Faculty of Pharmacy, Cairo University*, Faculty of Pharmacy, Cairo University, Vol. 54 No. 1, pp. 1–7.

Alhidayah, Fiqri, M. Al, Nirmayanti, Athiyyah, U., Fadjar, T.G.A., Nainu, F., Arjuna, A., *et al.* (2021), "Validation of spectrophotometric method for quantification of cefazoline in simulated tear fluid and porcine ocular tissue from thermosensitive-mucoadhesive in situ ocular gel preparation", *Chemical Data Collections*, Elsevier B.V., Vol. 36 No. November, p. 100793.

Ananda, P.W.R., Elim, D., Zaman, H.S., Muslimin, W., Tunggeng, M.G.R. and Permana, A.D. (2021), "Combination of transdermal patches and solid microneedles for improved transdermal delivery of primaquine", *International Journal of Pharmaceutics*, Elsevier B.V., Vol. 609 No. August, p. 121204.

Anjani, Q.K., Domínguez-Robles, J., Utomo, E., Font, M., Martínez-Ohárriz, M.C., Permana, A.D., Cárcamo-Martínez, Á., *et al.* (2022), "Inclusion complexes of rifampicin with native and derivatized cyclodextrins: In silico modeling, formulation, and characterization", *Pharmaceuticals*, Vol. 15 No. 1, available at:https://doi.org/10.3390/ph15010020.

Bacchetti, T., Morresi, C., Bellachioma, L. and Ferretti, G. (2020), "Antioxidant and pro-oxidant properties of Carthamus tinctorius, hydroxy safflor yellow A, and safflor yellow A", *Antioxidants*, Vol. 9 No. 2, available at:https://doi.org/10.3390/antiox9020119.

Das, S., Ng, W.K., Kanaujia, P., Kim, S. and Tan, R.B.H. (2011), "Formulation design,

preparation and physicochemical characterizations of solid lipid nanoparticles containing a hydrophobic drug: Effects of process variables", *Colloids and Surfaces B: Biointerfaces*, Vol. 88 No. 1, pp. 483–489.

Donnelly, R.F., Singh, T.R.R., Alkilani, A.Z., McCrudden, M.T.C., O'Neill, S., O'Mahony, C., Armstrong, K., *et al.* (2013), "Hydrogel-forming microneedle arrays exhibit antimicrobial properties: Potential for enhanced patient safety", *International Journal of Pharmaceutics*, Vol. 451 No. 1–2, pp. 76–91.

Dudhipala, N. and Gorre, T. (2020), "Neuroprotective effect of ropinirole lipid nanoparticles enriched hydrogel for parkinson's disease: In vitro, ex vivo, pharmacokinetic and pharmacodynamic evaluation", *Pharmaceutics*, Vol. 12 No. 5, pp. 1–24.

Enggi, C.K., Isa, H.T., Sulistiawati, S., Ardika, K.A.R., Wijaya, S., Asri, R.M., Mardikasari, S.A., *et al.* (2021), "Development of thermosensitive and mucoadhesive gels of cabotegravir for enhanced permeation and retention profiles in vaginal tissue: A proof of concept study", *International Journal of Pharmaceutics*, Elsevier B.V., Vol. 609 No. September, p. 121182.

Karimkhani, M.M., Shaddel, R., Khodaparast, M.H.H., Vazirian, M. and Piri-Gheshlaghi, S. (2016), "Antioxidant and antibacterial activity of safflower (Carthamus tinctorius L.) extract from four different cultivars", *Quality Assurance and Safety of Crops and Foods*, Vol. 8 No. 4, pp. 565–574.

Khalil, R.M., Abd-Elbary, A., Kassem, M.A., Ghorab, M.M. and Basha, M. (2014), "Nanostructured lipid carriers (NLCs) versus solid lipid nanoparticles (SLNs) for topical delivery of meloxicam", *Pharmaceutical Development and Technology*, Vol. 19 No. 3, pp. 304–314.

Khalil, R.M., El-bary, A.A., Kassem, M.A., Ghorab, M.M. and Ahmed, M.B. (2013), "Solid lipid nanoparticles for topical delivery of Meloxicam: development and In Vitro characterization", *European Scientific Journal*, Vol. 4 No. 7, pp. 24–26.

Kilic Buyukkurt, O., Guclu, G., Barutcular, C., Selli, S. and Kelebek, H. (2021), "LC-MS/MS fingerprint and simultaneous quantification of bioactive compounds in safflower petals (Carthamus tinctorius L.)", *Microchemical Journal*, Elsevier B.V., Vol. 171 No. September, p. 106850.

Kurkin, V.A. and Kharisova, A. V. (2014), "Flavonoids of carthamus tinctorius flowers", *Chemistry of Natural Compounds*, Vol. 50 No. 3, pp. 446–448.

Lademann, J., Richter, H., Schanzer, S., Knorr, F., Meinke, M., Sterry, W. and Patzelt, A. (2011), "Penetration and storage of particles in human skin: Perspectives and safety aspects", *European Journal of Pharmaceutics and Biopharmaceutics*, Elsevier B.V., Vol. 77 No. 3, pp. 465–468.

Mir, M., Permana, A.D., Tekko, I.A., McCarthy, H.O., Ahmed, N., Rehman, A. ur and Donnelly, R.F. (2020), "Microneedle liquid injection system assisted delivery of infection responsive nanoparticles: A promising approach for enhanced site-specific delivery of carvacrol against polymicrobial biofilms-infected wounds", *International Journal of Pharmaceutics*, Elsevier, Vol. 587, p. 119643.

Permana, A.D., Paredes, A.J., Volpe-Zanutto, F., Anjani, Q.K., Utomo, E. and Donnelly, R.F. (2020), "Dissolving microneedle-mediated dermal delivery of itraconazole nanocrystals for improved treatment of cutaneous candidiasis", *European Journal of Pharmaceutics and Biopharmaceutics*, Elsevier, Vol. 154 No. July, pp. 50–61.

Permana, A.D., Tekko, I.A., McCrudden, M.T.C., Anjani, Q.K., Ramadon, D., McCarthy, H.O. and Donnelly, R.F. (2019), "Solid lipid nanoparticle-based dissolving microneedles: A promising intradermal lymph targeting drug delivery system with potential for enhanced treatment of lymphatic filariasis", *Journal of Controlled Release*, Vol. 316, pp. 34–52.

Rostamkalaei, S.S., Akbari, J., Saeedi, M., Morteza-Semnani, K. and Nokhodchi, A. (2019), "Topical gel of Metformin solid lipid nanoparticles: A hopeful promise as a dermal delivery system", *Colloids and Surfaces B: Biointerfaces*, Elsevier, Vol. 175 No. October 2018, pp. 150–157.

De Souza, A.L.R., Andreani, T., Nunes, F.M., Cassimiro, D.L., De Almeida, A.E., Ribeiro, C.A., Sarmento, V.H.V., *et al.* (2012), "Loading of praziquantel in the crystal lattice of solid lipid nanoparticles: Studies by DSC and SAXS", *Journal of Thermal Analysis and Calorimetry*, Vol. 108 No. 1, pp. 353–360.

Üner, M., Karaman, E.F. and Aydoğmuş, Z. (2014), "Solid lipid nanoparticles and nanostructured lipid carriers of loratadine for topical application: Physicochemical stability and drug penetration through rat skin", *Tropical Journal of Pharmaceutical Research*, Vol. 13 No. 5, pp. 653–660.

Zhang, S., Qin, G., Wu, Y., Gao, Y., Qiu, Y., Li, F. and Xu, B. (2011), "Enhanced bioavailability of L-carnitine after painless intradermal delivery vs. oral administration in rats",

Pharmaceutical Research, Vol. 28 No. 1, pp. 117–123.

Zhang, Y., Yu, L., Jin, W., Li, C., Wang, Y., Wan, H. and Yang, J. (2020), "Simultaneous optimization of the ultrasonic extraction method and determination of the antioxidant activities of hydroxysafflor yellow A and anhydrosafflor yellow B from safflower using a response surface methodology", *Molecules*, Vol. 25 No. 5, pp. 1–15.

Zhu, R.R., Qin, L.L., Wang, M., Wu, S.M., Wang, S.L., Zhang, R., Liu, Z.X., *et al.* (2009), "Preparation, characterization, and anti-tumor property of podophyllotoxin-loaded solid lipid nanoparticles", *Nanotechnology*, Vol. 20 No. 5, available at:https://doi.org/10.1088/0957-4484/20/5/055702.

BUKTI ACCEPTED

Universitas Hasanuddin

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

Decision on Manuscript ID la-2022-02754m.R3

1 message

Langmuir <onbehalfof@manuscriptcentral.com> Tue, Jan 17, 2023 at 1:23 AM
Reply-To: heinz-office@langmuir.acs.org
To: andi.dian.permana@farmasi.unhas.ac.id

16-Jan-2023

Journal: Langmuir
Manuscript ID: la-2022-02754m.R3
Title: "Development of solid lipid nanoparticles loaded polymeric hydrogels containing antioxidant and photoprotective bioactive compounds of Safflower (Carthamus tinctorius L.) for improved skin delivery"
Authors: Aanisah, Nuur; Sulistiawati, Sulistiawati; Djabir, Yulia Yusrini; Asri, Rangga; Sumarheni, Sumarheni; Chabib, Lutfi; Hamzah, Hasyrul; Permana, Andi Dian
Manuscript Status: Accept

Dear Dr. Permana:

We are pleased to inform you that your manuscript has been accepted for publication in Langmuir.

You will soon receive an email invitation from the ACS Journal Publishing Staff that contains a link to the online Journal Publishing Agreement. Please sign and submit the journal publishing agreement within 48 hours.

Your manuscript has been forwarded to the ACS Publications office. You will be contacted in the near future by the ACS Journal Publishing Staff regarding the proofs for your manuscript.

After you approve your proofs, your manuscript will be published on the Web in approximately 48 hours. In view of this fast publication time, it is important to review your proofs carefully. Once a manuscript appears on the Web it is considered published. Any change to the manuscript once it appears on the Web will need to be submitted to the journal office as additions or corrections.

Once your paper is published, you can track downloads and citations of your work by logging into the ACS Publishing Center (https://pubs.acs.org/publish/dashboard) and selecting “Published”.

Langmuir has a twitter account (@ACS_Langmuir). If you would like us to tweet about your paper when it appears online, please recommend text for a tweet that is 200 characters or less (including spaces) to Langmuir Managing Editors (managing-editor@langmuir.acs.org) with your Manuscript ID as the subject line. You may include your twitter handle if you wish. We may modify the tweet before posting, and may not be able to include all tweets submitted. We appreciate your willingness to provide the tweet within one week of the receipt of this email.

Sincerely,

Prof. Hendrik Heinz
Senior Editor
Langmuir
University of Colorado at Boulder
Email:heinz-office@langmuir.acs.org

Thank you.