Pharmacy Education (2024) 24(2) 52 - 57
https://doi.org/10.46542/pe.2024.242.5257

IAI SPECIAL EDITION

RESEARCH ARTICLE

Formulation and evaluation of piperine-loaded ultra-small unilamellar carrier

Anugrah Putra Pharmaheri [1,2], Salman Umar [2], Henny Lucida [2]

[1]Dwi Farma Academy of Pharmacy Bukittingi, Bukittingi, Indonesia
[2]Faculty of Pharmacy, Universitas Andalas, Padang, Indonesia

Keywords
Emulgel
Liposome
Nanotope
Piperine
Ultra-small unilamellar carrier

Correspondence
Henny Lucida
Faculty of Pharmacy
Universitas Andalas
Padang
Indonesia
hennylucida@phar.unand.ac.id

Abstract

Background: Piperine is an alkaloid that has a stimulatory effect on the proliferation and dentricity of melanocytes. Therefore, it is a potential drug for vitiligo. **Objectives:** To formulate an Emulgel containing piperine-loaded Ultra-Small Unilamellar Carrier (USUC) with virgin coconut oil, soy lecithin and tween 80, and to evaluate the physicochemical properties. **Method:** Piperine-loaded USUC was formulated using a spontaneous water phase titration method at a stirring speed of 1500 rpm for 15 minutes with a base composition of 2.3% oil phase, 1% surfactant and 2.3% co-surfactant. Emulgel preparations were made by mixing USUC piperine into a 5% carbopol base. **Result:** Piperine-loaded USUC was obtained in the form of spherical globules with a Z-average size of 15.0 nm, a zeta potential of -39.5 mV and a polydispersion index of 0.312. The emulgel preparation has a pH of (6.5 ± 0.1), and a viscosity (of 44.800 ± 0.367) cP and is physically stable. Permeation of piperine from USUC emulgel follows zero-order kinetics (y = 0.130x-2.027; R2 = 0.9998) with a flux value of (3.408 ± 1.110) µg/cm2/hour, which is significantly different from permeation of non-USUC piperine gel (0.000 ± 0) µg/cm2/hour)(p <0.05). **Conclusion:** Piperine-loaded USUC in emulgel formulation produced nanosized spherical globules, indicating a significantly different amount of permeated piperine compared to conventional piperine gel.

Introduction

Piperine or (E,E)1-[5-(1,3-benzodioxol-5-yl)-1-oxo 2,4pentadienyl] is the main alkaloid found in black pepper (*Piper nigrum* L.; Piperaceae) (Parmar *et al*., 1997). This compound has low solubility but shows good permeability and is classified as class II according to the Biopharmaceutics Classification System (BCS) (Wdowiak *et al*., 2023).

Previous studies show that piperine stimulates melanocyte replication and induces the formation of melanocytic dendrites in vitro (Lin *et al*., 1999; Venkatasamy *et al*., 2004) so that it has the potential to treat vitiligo or hypopigmentation.

Vitiligo is an acquired skin pigmentation disorder characterised by the presence of well-defined milky white hypopigmented macules (Lotti & D'Erme, 2014). Even though the prevalence of this disease in the world is low (0.5 - 1%) it can affect the patient's quality of life and self-confidence (Feily, 2014). This is because the spread of white spots can occur on the face, hands, feet, and other parts such as the genitals, mucous membranes, or extensor areas. In addition, depigmentation of the face or hands causes vitiligo patients to feel embarrassed, plus a psychological burden due to negative stigma from some people (Choi *et al*., 2014).

As a potential drug for vitiligo, it is necessary to design a transdermal delivery system so that piperine can penetrate the skin barrier and reach the stratum basalis layer, where skin pigment is formed. Various techniques have been developed to enhance topical piperine delivery, including nanoemulsions (Ozkan *et al*., 2022) and self-emulsifying drug delivery systems (Shao *et al*., 2015; Zafar *et al*., 2021). The nanocarriers improved topical effectiveness by increasing the

stability and delivery to specific skin layers (Chin *et al*., 2016; Lucida *et al*., 2023).

Ultra-small Unilamellar Carrier (USUC) matrix is currently being developed as a nanocarrier system in the cosmetic field known as nanotope™. This system is characterised by having a much smaller droplet size (≤ 40nm) in comparison to other unilamellar or multilamellar liposomes (100-300) nm (Baschong *et al*., 2005). In the USUC, a dispersed phase is surrounded by a single phosphatidylcholine (surfactant) layer, stabilised by a co-surfactant in the dispersing medium. The smaller droplet size is beneficial in allowing the permeation of active ingredients through the skin to reach the lowest skin layer (stratum basalis), providing a better effect on the skin (Barel *et al*., 2018).

The USUC is a promising nanocarrier system for piperine because of much smaller globules with further improvement in permeability and efficacy. Formula optimisation of USUC by using a 2^5 factorial design has been developed (Chairunisa, 2022). The present study aims to formulate an emulgel containing Piperine-Loaded USUC (PLU) and to evaluate the physicochemical properties.

Methods

Materials

Piperine (Boc Sciences, USA), soya lecithin (food grade, Shankar Soya Products, Indonesia), Virgin Coconut Oil (VCO, Wahana, Indonesia), Tween 80 (Bratachem, Indonesia) were used. All other chemicals were of pro-analysis grade.

Design

Seven formulations of PLU were made using the USUC base from the authors' previous study with droplet size < 40 nm (Chairunnisa, 2022). PLU containing 1% piperine was prepared using spontaneous water phase titration method at a stirring speed, duration and USUC composition as described in the previous study. Emulgel preparations were made by mixing 1% PLU into a 5% carbopol base. Evaluation of emulgel preparations included pH, viscosity, percentage recovery, and permeation tests with Franz diffusion cells.

Assessments

Physicochemical properties

The pH of PLU was measured by using a pH meter (Voltcraft, Germany) which was previously calibrated. The measurement was done once a week during an eight-week storage at room temperature. The transmittance was measured by using a Spectrophotometer UV-Visible (SHIMADZU UV-1601, Japan) at a wavelength of 650 nm. A transmittance of close to 100% indicates the transparency of the liquid.

The physical stability of PLU was evaluated using a freeze-and-thaw cycling test. The USUCs were kept on storage at a temperature of -5 ^{o}C for 24 hours and then at 25 ^{o}C for another 24 hours. The test was repeated for three cycles.

The viscosity of PLU was measured by using a cup and bob viscometer (Brookfield DV2T, USA) using spindle number 3 at a speed of 100 rpm in triplicates, whereas the specific gravity was determined using a pycnometer at 25 ^{o}C.

Vesicular size analysis

Particle size and particle size distribution, polydispersity index (PDI), and zeta potential were measured by a particle size analyser (Horiba SZ 100). Samples were diluted using distilled water and placed in an electrophoretic cell.

Vesicle morphology

The morphology of PLU in emulgel was analysed by Transmission Electron Microscopy (TEM, JEOL 1010). The sample was placed on a TEM copper grid coated with a carbon film.

In-vitro permeation study by Franz diffusion apparatus

Validation of analytical method: The calibration curve was made by measuring the absorbances of piperine solution (concentrations 2, 3, 4, 5, and 6 µg/ml, respectively) at 342 nm using a UV-visible Spectrophotometer (Shimadzu UV-1601, Japan). A linear correlation was obtained ($y = 0.1277x + 0.0179$; $R2 = 0.9991$), with a Limit of Detection (LOD) of 0.1609 µg/mL and a Limit of Quantitation (LOQ) of 0.5364 µg/mL.

Permeation study: The emulgel permeation test was carried out using Whatman paper no.1 as a membrane. One gram of emulgel was weighed and smeared on a diffusion cell plate and then covered with the membrane. Fifteen mL of phosphate buffer solution pH 7.4 was put into the receptor compartment, placed in a water bath at 37 ± 2 °C, and stirred at 40 rpm. Five mL of samples were taken at 30, 60 and 120 minutes, respectively. A fresh buffer solution was added to the receptor solution after sampling. The cumulative amount of piperine phosphate permeated per diffusion area was calculated using the equation as follows (Thakker & Chern, 2003):

$$Q = \frac{C \times \frac{V_{medium}}{V_{sample}}}{A}$$

The flux was calculated using the equation as follows:

$$J = \frac{Q}{t}$$

Where: Q is the cumulative amount permeated per diffusion area (µg/cm2), C is concentration, V is volume, and A is membrane surface area. J is flux (µg /cm2/hour); t is time (hour).

Statistical analysis

Data are shown as mean ± standard deviation, and analysed by *t*-test statistical analysis to establish the significant differences between treatments.

Results

Seven formulations of PLU (F1-F6, and the optimum formula) with droplet size < 40 nm were prepared and evaluated. There were no changes in color, smell, or homogeneity after the freeze and thaw test for 3 cycles (Figure 1A). However, there were changes after the 8-week storage at room temperature for F1-F4, except for F5, F6 and the optimum formula, which remained stable (Figure 1B). The physicochemical properties of these formulations (Table I) show that the pH values of weak acidic solution (6.4-6.8) which is tolerable to the skin. The viscosity is in the range of 15.00–217.6 cP, which correlates with the concentration of tween 80. The specific gravity is in the range of 1.026-1.071 g/mL. The transmittance varied in the order of F3<F2<F4<F1<F6<F5<F-opt.

Figure 1: Piperine-loaded USUC (F1-F6 and F-opt) after 3 cycles of freeze and thaw test (A), and 8-week storage at room temperature (B)

Table I: The physicochemical properties of piperine-loaded USUC

Formula	pH	Viscosity (cP)	Specific gravity (g/mL)	Transmittant (%)	Physical stability
1	6.5±0.1	17.3± 0.5	1.031	87.47 ± 0.40	Not stable
2	6.8±0.1	15.0± 1.0	1.032	61.50± 0.45	Not stable
3	6.7±0.1	17.3± 0.5	1.026	35.48 ± 0.39	Not stable
4	6.8±0.1	16.0± 0.0	1.032	73.50 ± 0.45	Not stable
5	6.5±0.1	154.6±1.1	1.047	89.89 ± 0.75	Stable
6	6.5±0.1	217.6± 0.5	1.071	88.80 ± 0.76	Stable
Opt	6.4±0.0	38.0±0.0	1.055	90.54 ± 0.41	Stable

The stability characteristic of the optimum PLU was confirmed by the determination of zeta potential, polydispersity index values, and particle size distribution. Results showed homogeneously distributed vesicles (PDI = 0.312) with a Z-average size of 15.0 nm and zeta potential value of -39.5 mV. The addition of 1% PLU (F-opt) into the gel base resulted in a slightly opaque semi-solid dosage form with pH value (6.5 ± 0.1), viscosity (44.800 ± 0.367 cP), and recovery (100.91 ± 1.07)%. Morphological examination of PLU emulgel showed ultra-small unilamellar vesicles characterised by the formation of spherical globules dispersed in the gel base (Figure 2).

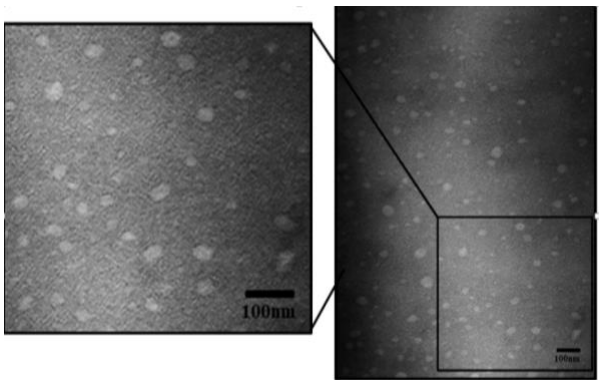

Figure 2: TEM image of piperin-loaded USUC emulgel (scale bar = 100.0 nm)

The permeation test results (Figure 3) showed an increase in the cumulative amount of piperine in the receptor medium released from the PLU gel, compared to that from a conventional gel containing microsized piperine in 5% carbopol. Permeation of piperine from USUC emulgel follows zero order kinetics (y = 0.13x-2.027; R2 = 0.9998) with the flux of 2.217; 3.591; and 4.415 µg/cm^2/hour at 30, 60, and 120 minutes respectively. The average flux value was (3.408 ± 1.110) µg/cm^2/hour which is significantly different from permeation of non-USUC piperine gel (0.000 ± 0) µg/cm^2/hour)(p <0.05).

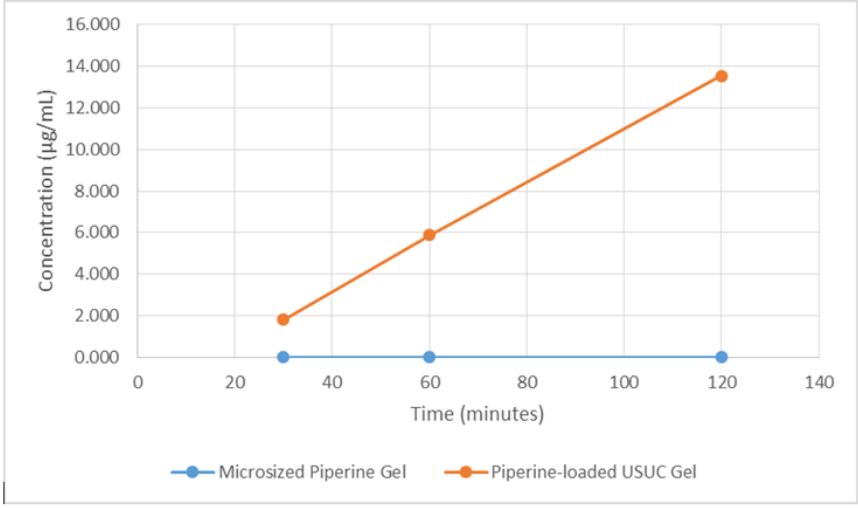

Figure 3: Concentration of piperine released from the USUC gel compared with microsized gel

Discussion

The authors' previous study demonstrates that the proportion of the oily phase: the aqueous phase : the stabilisers (soya lecithin-tween 80) and the manufacturing conditions (the stirring time and speed) greatly influenced the formation of nanosized vesicle as USUC base. The optimised conditions obtained from the study were 28.2% tween 80, 1% soya lecithin, 2.3% VCO at stirring speed 1500 RPM for 15 minutes (Chairunnisa, 2022). These conditions provide USUC base with a predicted vesicle size of 34.04 nm.

A single layer lipid core of USUC is composed of a phospolipid (soy lecithin) and a co-surfactant (tween 80) at a fixed ratio. The co-surfactant (tween 80) intercalates between the soy lecithin molecules, which is stabilised by van der Waals hydrophobic interaction forces (Ghanbarzadeh *et al*., 2016).

The incorporation of piperine into the USUC base produces a transparent yellow solution. All formulations were stable after the freeze and thaw tests, but F1-F4 were unstable when stored for 8 weeks at room temperature. These results indicate that extreme temperature changes in the freeze and thaw test do not affect the stability of piperine in nanometer-sized vesicles of the USUC system, but longer storage time at room temperature affects the stability of 4 formulas. Further study is needed to evaluate the stability of PLU in long-term storage at room temperature.

The transmittance of the USUC base was varied: high transmittance values indicate the clarity of the solution and correlate with smaller droplet sizes. Nanosized particles scatter incoming light which is demonstrated by opalescence appearance (Kallay & Žalac, 2002). Surfactants may intercalate into the phospholipid bilayer and induce vesicle disruption to obtain a clearer solution, even though a stable vesicle should maintain a light-scattering nature (Baschong *et al*., 2005). The optimum formula of PLU had a transmittance of (90.54 ± 0.41)%, which showed a transparent solution and remained stable after storage.

The droplet size of PLU (15.0 nm) was much smaller than the optimum USUC base (34.04 nm) with moderate stability against agglomeration as indicated by zeta potential of -39.5 mV and homogeneously distributed (0.2 < PDI < 0.5) (Đorđević *et al*., 2017). The photomicrograph of PLU emulgel showed spherical globules surrounded by a thin layer membrane. Unilamellar appearance is usually indicated by a

transparent layer surrounding the globules, which are not clearly visible in Figure 2. This distinguishes nanotopes, which are ultra-small unilamellar vesicles, from large multilamellar liposomes whose vesicles are surrounded by thick transparent layers (Baschong *et al*., 2005).

Kinetic analysis of the concentration of piperine permeating per unit of time followed zero-order kinetics. The kinetic profile indicates that the transport of piperine into the medium is most likely through membrane pores due to its very small size (15 nm), which fits the zero-order kinetics. This also explains why the microsized piperine in the conventional gel can not transport through the membrane. Zafar *et al*. (2021) reported a 9.32-fold increase in piperine permeation from the self-nanoemulsifying drug delivery system (SNEDDS) compared to pure piperine dispersion. The higher permeation was due to the higher solubilisation of piperine in the SNEDDS. The release profile of piperine from the SNEDDS has also fitted the zero-order kinetics and found to obey the Fickian diffusion mechanism (n=0.2096) (Zafar *et al*., 2021).

Conclusion

PLU with a composition of 1% piperine, 2.3% VCO, 1% lecithin and 28.3% tween 80 produced spherical vesicles that were homogeneously distributed (PDI=0.312) with the Z-average size of 15.0 nm and a zeta potential of -39.5 mV. Emulgels containing 1% PLU showed a significant increase in the amount of piperine permeated compared to microsized piperine gels (p <0.05).

Acknowledgement

The authors would like to thank Prof. Dr. apt. Erizal Zaini for his kindness in providing piperine for this research. This article was presented at the 2023 Annual Scientific Conference of the Indonesia Pharmacist Association.

References

Barel, A. O., Paye, M., & Maibach, H. I. (2018). *Handbook of cosmetic science and technology*. CRC Press.

Baschong, W., Herzog, B., Artmann, C. W., Mendrok, C., Mongiat, S., & Lupia, J. A. (2005). *Nanotopes^{TM}: A novel ultra-small unilamellar carrier system for cosmetic actives.* William Andrew Inc.

Chairunisa, U., Rustini, Nastiti, C. M. R. R., Riswanto, F. D. O., Benson, H. A. E., & Lucida, H. (2022). A promising ultra-small unilamellar carrier system for enhanced skin delivery of α-mangostin as an anti-age-spot serum. *Pharmaceutics*, **14**(12), 2741. https://doi.org/10.3390/pharmaceutics14122741.

Chin, G. S., Todo, H., Kadhum, W. R., Hamid, M. A., & Sugibayashi, K. (2016). In vitro permeation and skin retention of α-mangostin proniosome. *Chemical and Pharmaceutical Bulletin*, **64**(12), 1666–1673, https://doi.org/10.1248/cpb.c16-00425.

Choi, D., Isedeh, P., & Hamzavi, I. (2014). Vitiligo: A review of the pathogenesis. *Journal of the Egyptian Women's Dermatologic Society*, **11**(3), 145–158. https://doi.org/10.1097/01.EWX.0000450307.76457.a3.

Đorđević, S. M., Santrač, A., Cekić, N. D., Marković, B. D., Divović, B., Ilić, T. M., Savić M. M., & Savić, S. D. (2017). Parenteral nanoemulsions of risperidone for enhanced brain delivery in acute psychosis: Physicochemical and in vivo performances. *International Journal of Pharmaceutics*, **533**(2), 421–430. https://doi.org/10.1016/j.ijpharm.2017.05.051.

Feily, A. (2014). Vitiligo Extent Tensity Index (VETI) score: A new definition, assessment and treatment evaluation criteria in vitiligo. *Dermatology Practical & Conceptual*, **4**(5), 81–84. https://doi.org10.5826/dpc.0404a18.

Ghanbarzadeh, B., Babazadeh, A., & Hamishehkar, H. (2016). Nano-phytosome as a potential food-grade delivery system. *Food Biosciences,* **15**, 126–135, https://doi.org/10.1016/j.fbio.2016.07.006.

Kallay, N., & Žalac, S. (2002). Stability of nanodispersions: A model for kinetics of aggregation of nanoparticles. *Journal of Colloidal Interface Sciences*, **253**(1), 70–76, https://doi.org/10.1006/jcis.2002.8476.

Lin, Z., Hoult, J. R., Bennett, D. C., & Raman, A. (1999). Stimulation of mouse melanocyte proliferation by *Piper nigrum* fruit extract and its main alkaloid, piperine. *Planta Medica*, **65**(7), 600–603. https://doi.org/10.1055/s-1999-14031.

Lotti, T., & D'Erme, A. M. (2014). Vitiligo as a systemic disease. *Clinics in Dermatology*, **32**(3), 430–434. https://doi.org/10.1016/j.clindermatol.2013.11.011.

Lucida, H., Hasani, S., Susanti, M., & Ismed, F. (2023). Formulation of a gambier catechin-loaded nanophytosome and the MTT assay on HeLa celllines. *Pharmacy Education*, **23**(2), 19–24. https://doi.org/10.46542/pe.2023.232.1924.

Ozkan, B., Alluntas, E., Koc, R. C., & Budama-Kilinc, Y. (2022). Development of piperine nanoemulsions: An alternative topical application for hypopigmentation. *Drug Development and Industrial Pharmacy*, **48**(3), 117–127. https://doi.org/10.1080/03639045.2022.2100901.

Parmar, V. S., Jain, S. C., Bisht, K. S., Jain, R., Taneja, P., Jha A., Tyagi, O. D., Prasad, A., Wengel, J., Olsen, C. E., & Boll, P. M. (1997). Phytochemistry of genus Piper. *Phytochemistry*, **46**(4), 597–673. https://doi.org/10.1016/S0031-9422(97)00328-2

Shao, B., Cui, C., Ji, H., Tang, J., Wang, Z., Liu, H., Qin, M., Li, X., & Wu, L. (2014). Enhanced oral bioavailability of piperine by self-emulsifying drug delivery systems: In vitro, in vivo and in situ intestinal permeability studies. *Drug Delivery*, **22**(6), 740–747. https://doi.org/10.3109/10717544.2014.898109.

Thakker, K. D., & Chern, W. H. (2003). Development and validation of in vitro release tests for semisolid dosage forms—Case study. *Dissolution Technologies*, **10**(2), 10–15. https://doi.org/10.14227/DT100203P10.

Zafar, A., Imam, S. S., Alruwaili, N. K., Alsaidan, O. A., Elkomy, M. H., Ghoneim, M. M., Alshehri, S., Ali, A. M. A., Alharbi, K. S., & Yasir, M. (2021). Development of piperine-loaded solid self nanoemulsifying drug delivery system: Optimization, in-vitro, ex-vivo, and in-vivo evaluation. *Nanomaterials*, **11**(11), 2920. https://doi.org/10.3390/nano11112920

Venkatasamy, R., Faas, L., Young, A. R., Raman, A., & Hider, R. C. (2004). Effects of piperine analogues on stimulation of melanocyte proliferation and melanocyte differentiation. *Bioorganic and Medicinal Chemistry*, **12**(8), 1905–1920. https://doi.org/10.1016/j.bmc.2004.01.036.

Wdowiak, K., Miklaszewski, A., Pietrzak, R., & Cielecka-Piontek, J. (2023). Amorphous system of hesperetin and piperine-improvement of apparent solubility, permeability, and biological activities. *International Journal of Molecular Sciences*, **24**(5), 4859. https://doi.org/10.3390/ijms24054859.