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Development, characterization, molecular docking, and *in vivo* skin penetration of coenzyme Q10 nanostructured lipid carriers using tristearin and stearyl alcohol for dermal delivery

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Abstract

Objectives: This study aims to develop coenzyme Q10 nanostructured lipid carriers (NLCs) using tristearin and stearyl alcohol as well as isopropyl palmitate (IPP) as solid and liquid lipid respectively for the dermal delivery system.
Methods: The coenzyme Q10 NLCs were optimized using tristearin, and stearyl alcohol in different concentrations and further characterized by dynamic light scattering (DLS) for particle size, polydispersity index (PDI), zeta potential, differential scanning calorimetry (DSC) and X-ray diffractometry for crystallinity behavior, Fourier transform infrared spectroscopy (FT-IR) for drug-lipid interaction, scanning electron microscopy (SEM) for particle shape, viscometer for viscosity, and pH meter for pH value. Furthermore, entrapment efficiency (EE), drug loading (DL), and skin penetration *in vivo* were also evaluated while molecular docking was conducted to examine the interaction between coenzyme Q10 and the lipids.
Results: The coenzyme Q10 NLCs with tristearin-IPP and stearyl alcohol-IPP as lipid matrix had <1,000 nm particle size, <0.3 PDI, less negative than −30 mV zeta potential, about 41% crystallinity index, and about six as the pH value. Moreover, the EE, DL, viscosity, and *in vivo* skin penetration of the NLCs using tristearin were higher compared to stearyl alcohol, however, the skin penetration depths for both NLCs were not significantly different. Furthermore, the *in silico* binding energy of coenzyme Q10-tristearin was lower compared to coenzyme Q10-stearyl alcohol. Both of them showed hydrophobic and van der Waals interaction.
Conclusions: The NLCs of coenzyme Q10 were formulated successfully using tristearin-IPP and stearyl alcohol-IPP for dermal delivery.

Keywords: coenzyme Q10; dermal delivery; molecular docking; NLC; skin penetration.

Introduction

Coenzyme Q10 is a lipid-soluble antioxidant due to the 10 isoprenoid side-chains. Chemically, it is known as 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone [1]. Furthermore, coenzyme Q10 is used for skin anti-aging in cosmetic products. However, the skin penetration is poor due to its lipophilic property and large molecular weight (863.36 g/mol) [2–5]. The nanodelivery system is potentially used to overcome this problem since it enhances dermal penetration due to its active ingredients, lipid nanoparticles constitute part of this system [6, 7].

The two main classes of lipid-based nanoparticles are solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), the difference between both categories depends on the lipid matrix. For SLNs, only solid lipids are used whereas the lipids matrix for NLCs consisted of solid and liquid lipids. Due to the incorporation of liquid lipids in NLC, the crystal structure arrangement of the solid lipid become disordered hence, the entrapment efficiency (EE), drug loading (DL), and stability of NLC increases [6, 8].

In this study, coenzyme Q10 NLCs were developed using tristearin and stearyl alcohol as solid lipids and isopropyl myristate (IPM) or isopropyl palmitate (IPP) as liquid lipids. Meanwhile, tristearin and stearyl alcohol have different lipophilicity with the former being more lipophilic than the latter [9]. IPM or IPP is considered as a skin penetration enhancer [10].

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Therefore, this study aims to develop the coenzyme Q10 NLCs for dermal delivery using tristearin and stearyl alcohol as solid lipids and IPM or IPP as liquid lipids. The NLC formulas were optimized to obtain optimal coenzyme Q10 NLCs for dermal delivery thereafter, the coenzyme NLCs were evaluated in physicochemical characteristics and *in vivo* skin penetration through rats' skin. Moreover, *in silico* studies via molecular docking were also conducted to elucidate the interactions between coenzyme Q10 and solid lipid.

Materials and methods

Materials

The coenzyme Q10 was purchased from Kangcare Bioindustry Co., ltd. Nanjing, China while Tristearin analytical grade was purchased from Sigma Aldrich (St. Louis, MO, USA). Also, Stearyl alcohol, Span 80, phenoxyethanol were purchased from Universal Pharma Chemical (Surabaya, Indonesia) while IPM, IPP, propylene glycol, and Tween 80 were purchased from Bratachem (Surabaya, Indonesia). Furthermore, Ethanol 96%, NaH_2PO_4, and Na_2HPO_4 (analytical grade) were purchased from E.Merck (Darmstadt, Germany). All materials used in the study fulfilled pharmaceutical-grade unless otherwise stated.

Preparation of optimized coenzyme Q10 NLCs formulas

Using the high shear homogenization method, the lipids were melted at 80 °C and stirred at 3,400 rpm with ultra turrax for 1 min until a homogeneous mixture was obtained. The 1% Coenzyme Q10 was placed into the mixture and agitated for about 2 min until dissolved. Furthermore, the 2% Span 80 and 18% Tween 80 were heated to 80 °C separately and poured gradually while the 10% propylene glycol and 69% phosphate buffer were also heated to 80 °C and then poured into the lipid phase and stirred until homogeneous for about 1 min, thereafter, the stirring speed was increased to 24,000 rpm for 3 min, at 3,400 rpm, 0.6% phenoxyethanol was added at 40 °C and stirred continuously until room temperature. The coenzyme Q10 NLCs formulations for optimization are shown in Table 1

Table 1: The coenzyme Q10 NLCs formulas for optimization (concentration materials in %).

Materials	Lipid 8%			Lipid 10%			Lipid 15%
	F1	F2	F3	F4	F5	F6	F7
Coenzyme Q10	1	1	1	1	1	1	1
Tristearin	5.6	–	–	7	–	–	–
Stearyl alcohol	–	5.6	5.6	–	7	7	10.5
IPM	–	2.4	–	–	3	–	–
IPP	2.4	–	2.4	3	–	3	4.5

NLC, nanostructured lipid carriers; IPP, isopropyl palmitate; IPM, isopropyl myristate.

Particle size, polydispersity index (PDI), and zeta potential

The nanoparticle analyzer (Nanotrac Wave, Microtrac W3717) was used to measure particle size, PDI, and zeta potential of the coenzyme Q10 NLCs. Meanwhile, before the test, the samples were diluted with appropriate aqua dem.

Thermal behaviors

Thermal behaviors for coenzyme Q10, the solid lipids, and coenzyme Q10 NLCs were analyzed using differential scanning calorimetry (DSC). Approximately, 4 mg of the samples were heated from 30 to 100 °C in a calorimeter (DSC model 1/500, Mettler Toledo) with a heating rate of 10 °C/min. The percentage of crystallinity index (%CI) was measured using the following equation:

$$\%CI = \frac{\text{enthalpy } (\Delta H) \text{ coenzyme Q10 NLC}}{\Delta H \text{ lipid matrix} \times \text{concentration lipid phase}} \times 100$$

The X-ray diffraction

Crystallinity behaviors and X-ray diffractions were evaluated using an X-ray diffractometer (Phillip X'pert). The samples were analyzed in 2θ range of 4–40° at 40 kV, 30 mA.

Fourier transform infrared

Drug-lipid interaction was determined using Fourier transform infrared (FT-IR) spectra. The samples in a KBr were prepared to form a pellet and then scanned at wavelengths of 400–4,000 cm^{-1} using FT-IR Spectrophotometer (Jasco FT-IR 5300).

Morphology

The coenzyme Q10 NLCs morphology was evaluated using scanning electron microscopy (SEM, ZEISS) with 25,000× magnification.

Rheology and viscosity

The Cone and Plate viscosimeter (Brookfield AT 17362, spindle CPE-41) were used to determine the rheology and viscosity of the coenzyme NLCs.

The pH value

The calibrated pH meter (SI analytic LAB 850) was utilized for evaluating the pH value of NLCs.

The entrapment efficiency (EE) and drug loading (DL)

The indirect method was used to assign the EE and the DL of coenzyme Q10 NLCs. The free coenzyme Q10 was obtained through centrifugation of coenzyme Q10 NLC at 10,000 rpm for 30 min. Prior to this, the

former was diluted with a known amount of aqua dem. The filtrate absorbance was measured using a spectrophotometer UV1800 (Shimadzu) at a wavelength of 275 nm.

In silico studies by molecular docking

The two and three-dimensional (2D and 3D) chemical structures of coenzyme Q10, tristearin, and stearyl alcohol were generated using ChemDraw® Pro 2016 (Cambridgesoft), as well as the energy minimization process using the same program. Also, molecular docking of coenzyme Q10 and solid lipids were performed by Autodock Tools (ADT)1.5.6, and AutoDock Vina (The Scripps research group) while the docking results were visualized with Discovery Studio Visualizer (DSV) (Biovia) and ADT.

Skin penetration *in vivo*

Skin penetration *in vivo* was performed using male Wistar rats weighing 200–300 g, aged 6–8 weeks without defects nor skin disease, also, there were no wounds after cleaning the rat's hair. Thereafter, the coenzyme Q10 NLC with Nile red as a fluorescent label was spread on the skin of the hairless rats which were split into three groups. The first, second, and third groups were sacrificed via cervical dislocation at 2, 4, 6 h respectively after Q10 NLC application. Furthermore, the rats' skin was made into histological preparations using a frozen cryotome while the depth of penetration was measured using fluorescence microscopy (Olympus FX-1000). The experimental animals were used with permission from the Animal Care and Use Committee (ACUC), Airlangga University (ethical clearance No. 2.KE.174.09.2019).

Statistical analysis

The Student's t-test was used for evaluating the differences in mean values (n=3) of the physicochemical characteristics with $p<0.05$, whereas in the *in vivo* studies of rats skin penetration, the two-way ANOVA method with $p<0.05$ was used to determine the differences in mean values (n=3) among the formulation groups.

Results

Particle size, polydispersity index (PDI), and zeta potential

The Coenzyme Q10 NLCs formulas with different lipid matrix concentrations were evaluated to obtain optimal NLCs. The coenzyme Q10 NLCs had particle size 472–1,063 nm, PDI 0.297–0.293, and zeta potential −11.5 to −20.3 mV. The particle size of (F1) and (F3) were 472.0 ± 47.1 and 684.3 ± 8.0, respectively, hence, the particle size for (F1) was smaller than (F3) whereas, (F2), (F4), (F5), (F6), and (F7) were >600 nm.

Thermal behaviors

The melting point and enthalpy of coenzyme Q10, tristearin, stearyl alcohol, coenzyme Q10 NLC (F1), and (F3) showed endothermic peaks as presented in Figure 1 and Table 2. The %CI of solid lipids, coenzyme Q10 NLC (F1), and (F3) were 100, 41.47, and 41.44%, respectively.

The X-ray diffraction

The X-ray diffraction patterns for tristearin had several sharp peaks at 2θ values of 6.113, 21.117, and 23.293°, stearyl alcohol at 2θ values of 20.964 and 24.139° whereas,

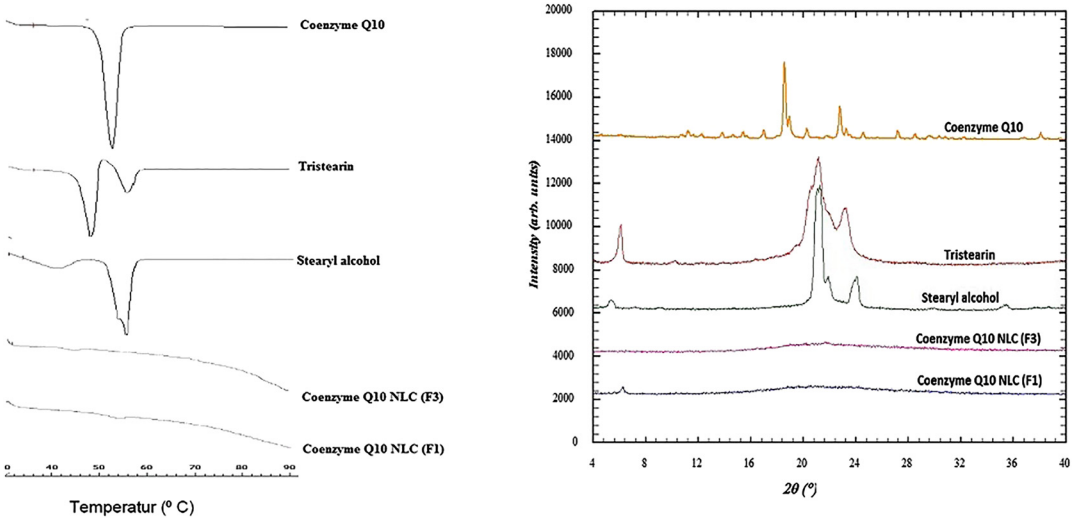

Figure 1: (A) DSC thermogram and (B) X-ray diffraction of coenzyme Q10, tristearin, stearyl alcohol, and coenzyme Q10.

Coenzyme Q10 had sharp peaks at 2θ values of 18.544 and 22.746° as presented in Figure 1.

Fourier transform infrared

FT-IR spectra of coenzyme Q10 compared to coenzyme Q10 NLCs, and the lipids at 4,000–400 m^{-1} are shown in Figure 2. The FT-IR spectra of coenzyme Q10 indicated peaks at 2,962.13, 1,732.73, 1,645.95, and 1,200.47 cm^{-1} for C–H, C=O, C=C, and C–O stretching respectively.

Morphology

Morphologically, the coenzyme Q10 NLC (F1), and (F3) showed spherical particles as presented in Figure 3.

Table 2: Melting point, enthalpy (ΔH), crystallinity index (CI) of coenzyme Q10, tristearin, stearyl alcohol, and coenzyme Q10 NLCs.

Materials	Melting point, °C		Enthalpy, J/g		CI, %
	Peak 1	Peak 2	Peak 1	Peak 2	
Coenzyme Q10	51.63	–	−153.2	–	–
Tristearin	48.24	56.77	−125.51	−43.91	100
Coenzyme Q10 NLC (F1)	–	55.49	–	−3.8	41.47
Stearyl alcohol	41.17	55.58	30.57	−162.29	100
Coenzyme Q10 NLC (F3)	–	44.31	–	−5.38	41.44

NLC, nanostructured lipid carriers; CI, crystallinity index.

Rheology and viscosity

The rheology was determined by examining the viscosity of coenzyme Q10 NLC (F1), and (F3) at various shear rates. The coenzyme Q10 NLCs viscosity decreased by increasing the rate of shear, as shown in Figure 4. In addition, the viscosity of the NLC Coenzymes Q10 (F1), and (F3) were 23,582 ± 1,922 and 17,739 ± 1,126 Cps at 0.5 rpm indicating that (F1) was higher than (F3) ($p<0.05$).

The pH value

The pH value of coenzyme Q10 (F1), and (F3) NLC were 5.88 ± 0.03 and 5.75 ± 0.10, respectively meanwhile, the pH values of the coenzyme Q10 NLC (F1) and (F3) were not significantly different ($p>0.05$).

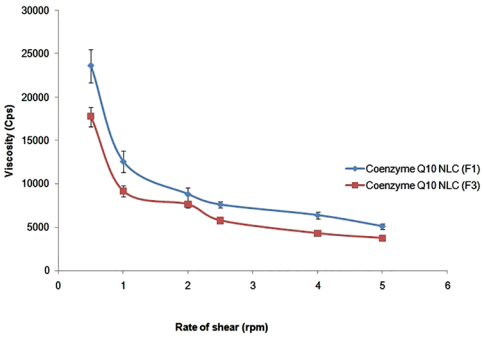

Figure 4: Rheology of coenzymeQ10 NLCs.

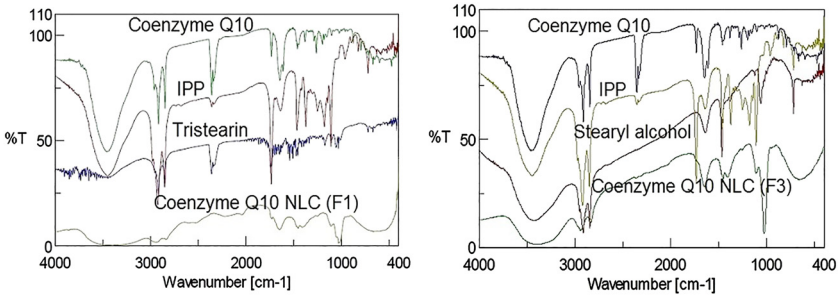

Figure 2: FT-IR spectra of coenzyme Q10, IPP, tristearin, stearyl alcohol, and coenzyme Q10 NLCs.

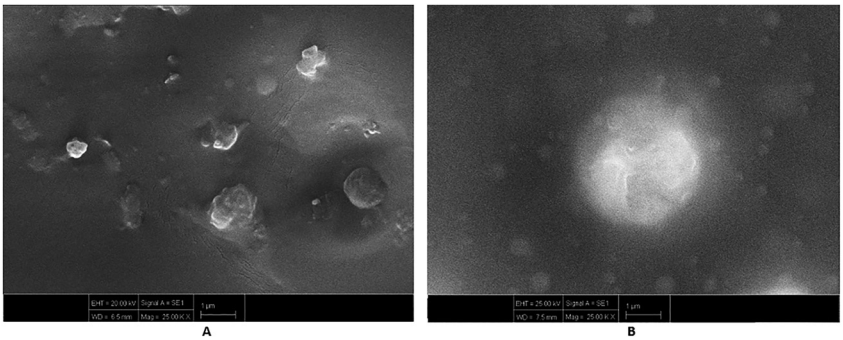

Figure 3: SEM image of (A) coenzymeQ10 NLC (F1) and (B) coenzymeQ10 NLC (F3) with a magnification of 25,000×.

The entrapment efficiency (EE) and drug loading (DL)

The EE of coenzyme Q10 NLC (F1) and (F3) were 90.612 ± 0.908 and 86.138 ± 1.786%, respectively whereas, the DL for both NLCs were 11.327 ± 0.113 and 10.767 ± 0.223%, respectively, indicating higher % EE and % DL values for (F1) compared to (F3) ($p<0.05$).

In silico studies by molecular docking

The binding energy (ΔG) *in silico* of coenzyme Q10-tristearin and coenzyme Q10-stearyl alcohol were −9.2 and −6.6 kcal/mol, respectively. Both of them showed hydrophobic and van der Waals interaction in the 3D visualization using DSV and ADT respectively as shown in Figure 5. Furthermore, this hydrophobic bond was at C18 atoms of tristearin and C48 atoms of coenzyme Q10 with a distance of 4.31 Å. A similar bond was found between coenzyme Q10 and stearyl alcohol at C18 atom of tristearin and C53 atom of coenzyme Q10 with a distance of 4.48 Å.

In vivo skin penetration

The skin penetration depth of coenzyme Q10 NLC (F1) at 2, 4, and 6 h after applications were 298.45 ± 8.70, 332.94 ± 36.27, and 358.34 ± 15.86 µm, respectively whereas, for (F3) with similar intervals, the obtained values were 294.64 ± 38.21, 340.73 ± 10.13, and 349.62 ± 6.59 µm. Hence, the depth of skin penetration for both (F1) and (F3) increased with increasing time ($p<0.05$) and showed no significant difference after 6 h ($p>0.05$). The fluorescence intensity of (F1) was higher compared to (F3), and however, both NLCs could across the stratum corneum and penetrated the skin, as shown in Figure 6.

Discussion

The Coenzyme Q10 NLC was designed to overcome coenzyme Q10 difficulties in penetrating the skin. Foremost, the coenzyme Q10 NLC formulas were optimized and then prepared using the high shear homogenization method with different concentrations and types of lipid matrix as shown in Table 1. Meanwhile, 1% Coenzyme Q10 in NLC was used due to its ability to induce the fibroblast cells in the mice model [11].

The lipid matrix concentrations include 8, 10, and 15%, while the ratio of solid to liquid lipid was 70:30. hence, the results showed that the lower the matrix concentration, the smaller the particle size. This is because, lower lipid matrix concentration requires less energy to reduce the particle size compared to higher lipid concentrations which increase the viscosity of the system but decrease the shearing capacity of the stirrer, hence, particle size reduction becomes difficult. Moreover, the surfactant concentration was not adequate to match the increasing lipid matrix concentration to cover the particle surface therefore, the particle size increased. The result was in line with other previous studies [12, 13].

The particle sizes for coenzyme Q10 NLC (F1) and F3 were ≤600 nm, whereas, others were >600 nm. Transdermal preparations have particle sizes of about 600 nm [14]. While particle size distribution of the coenzyme Q10 NLC (F1) and (F3) were homogenously dispersed (PDI < 0.3).

The Zeta potentials for (F1) and (F3) were less negative than −30 mV. Even though the zeta potential of NLCs Q10 was less negative than −30 mV, it did not mean physically unstable. Due to the use of nonionic surfactants (Tween 80, and Span 80), these compounds provide steric stability to the system [15]. It failed to ionize into a charged group such as ionic surfactants capable of producing zeta potential due to molecular polarization and constructed electric double-layer [16–18].

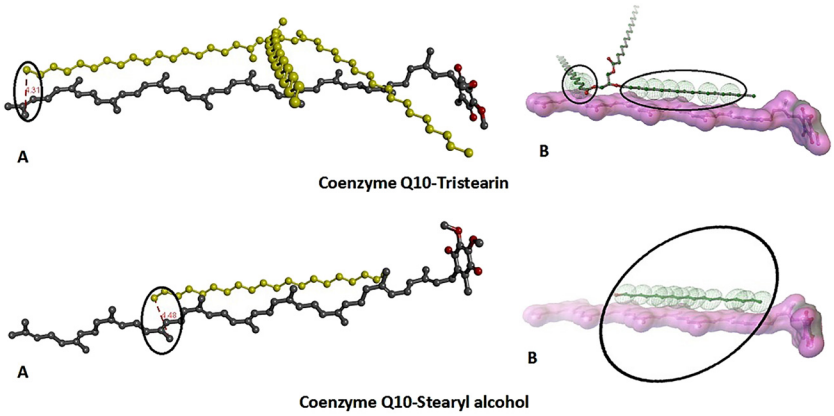

Figure 5: Molecular docking of coenzyme Q10 with tristearin and stearyl alcohol (A) using DSV show hydrophobic bonds and (B) using ADT show van der Waals interactions.

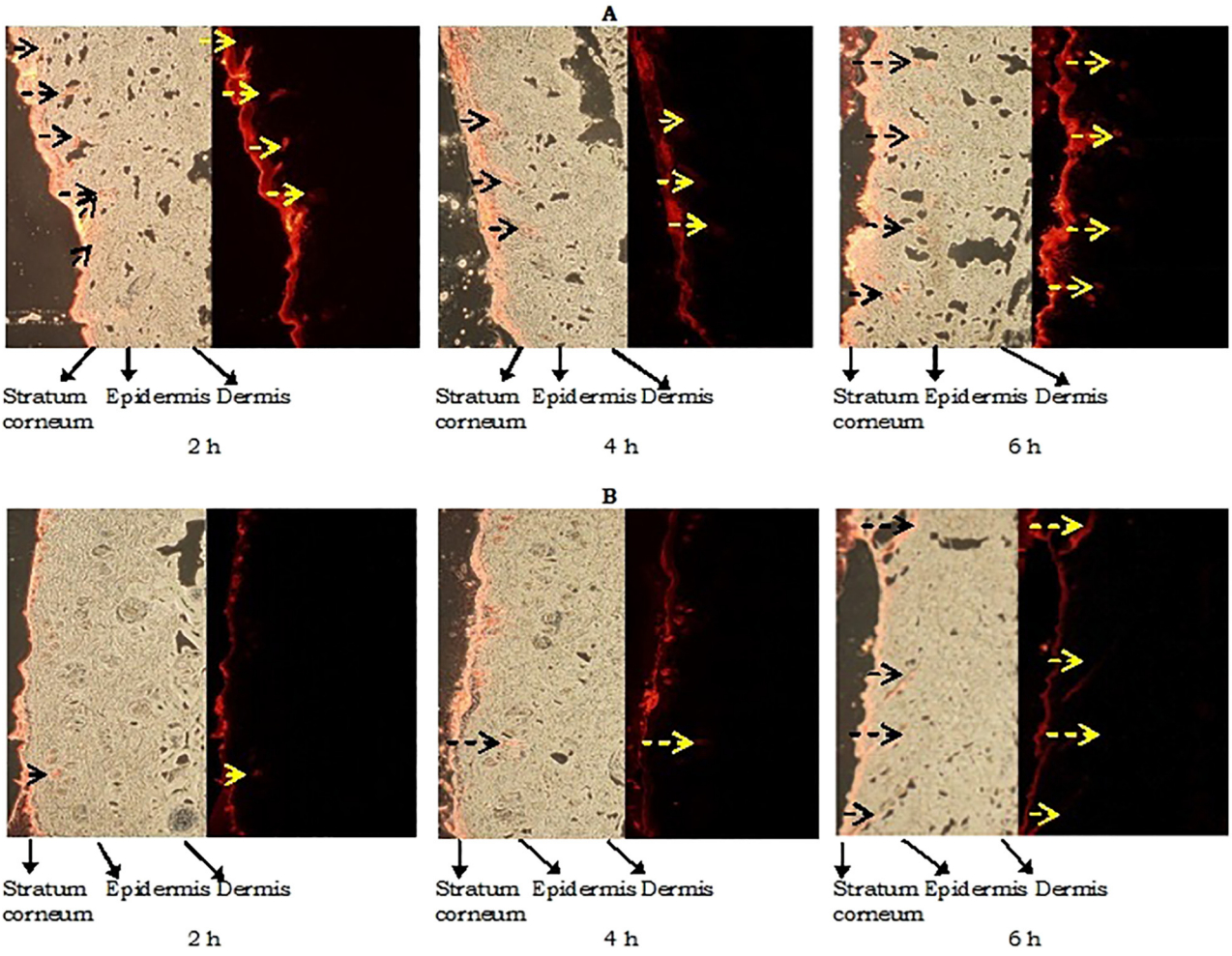

Figure 6: *In vivo* skin penetration through rats skin of (A) coenzyme Q10 NLC (F1) and (B) coenzyme Q10 NLC (F3) for 2, 4, and 6 h after applying the coenzyme Q10 NLC using a fluorescence microscope with a magnification of 10×.

Furthermore, the coenzyme Q10 NLC (F1) and (F3) were evaluated for physicochemical characteristics and *in vivo* percutaneous penetration through the rats' skin.

DSC was used to determine the physical characteristics and crystal structure of coenzyme Q10, solid lipids, and coenzyme Q10 NLCs [19]. Meanwhile, the DSC thermogram of tristearin and stearyl alcohol as presented in Figure 1 showed two endothermic peaks, indicating the presence of a crystal structure in tristearin and stearyl alcohol [20, 21]. In general, triglycerides and fatty acids exhibit three different polymorphs [22–24].

The melting point and enthalpy of coenzyme Q10 NLCs were lower compared to that of coenzyme Q10 and solid lipids, as shown in Figure 1 and Table 2. This was caused by the incorporation of IPP as liquid lipid hence, the crystal order of solid lipid became less ordered or amorf state in NLCs. Therefore, coenzyme Q10 was entrapped or dissolved in the lipid matrix [25, 26]. The CI of coenzyme Q10 NLCs were determined by assuming the CIs of solid lipids were 100%, meanwhile, this value decreased compared to the solid lipid. Furthermore, the CI of coenzyme Q10 NLC (F1) and (F3) were less than 50%, as shown in Table 2 indicating that the coenzymes NLCs were in an amorphous state hence, creating enough space for the entrapment of coenzyme Q10 [25–27].

In this study, the DSC analysis was combined with the XRD. Furthermore, the X-ray diffraction pattern was employed to support the crystallinity analysis of the molecules [19]. The X-ray diffraction pattern of coenzyme Q10, tristearin, and stearyl alcohol presented sharp peaks, as shown in Figure 2 whereas, the peak intensity of the coenzyme Q10 NLCs decreased. Meanwhile, sharp peaks point to a highly crystallized particle state [28]. The decreased intensity of the coenzyme Q10 NLCs indicated

that the coenzyme Q10 was entrapped or dissolved in the lipid matrix in an amorphous state [29–31].

The FT-IR spectra of coenzyme Q10 were closely in line with the previous study [32]. There was no significant shift in wavenumber and new peaks for coenzyme Q10 NLCs compared to coenzyme Q10 and lipid using the FT-IR spectra. However, there was a burned peak at 1,732.73 nm, which correlates with functional group C=O stretching in coenzyme Q10. These results indicated that coenzyme Q10 was entrapped in the lipid matrix, and the absence of chemical interaction between coenzyme Q10 and lipid matrix which capable of changing the wavenumber and creating new peaks. Similar results were presented in another study with different drugs and lipid matrix [33–36].

The coenzyme Q10 NLC (F1) had an almost spherical shape while F3 was completely spherical. Meanwhile, an aggregate of molecules appeared prior to SEM analysis with a sticky lipid matrix.

The flow behavior for coenzyme Q10 (F1), and (F3) NLC were non-Newtonian, pseudoplastic due to the decrease in viscosity with increasing shear rate [37] as presented in Figure 4. Therefore, the viscosity of coenzyme Q10 NLC (F1), and (F3) depended on the rate of shear. Both coenzyme NLCs had semisolid consistencies which were suitable for dermal application due to the ease in dispersibility on the skin.

The pH values of the coenzyme NLCs were suitable for dermal preparations as they were similar to the pH of the skin (4–6.5) [38].

The EE and DL of coenzyme Q10 NLC (F1) higher than (F3). This is because coenzyme Q10 is a lipophilic substance with lop p>10 (5), therefore it was more soluble in tristearin compared to stearyl alcohol. Also, the lipophilicity of tristearin, as solid lipid in coenzyme Q10 NLC (F1), was higher compared to stearyl alcohol. Apart from its crystallinity behaviors, the lipophilicity of the lipid matrix also influences EE and NLC drug loading [39, 40].

In silico molecular docking between coenzyme Q10 and solid lipids showed that the binding energy (ΔG) coenzyme Q10-tristearin was lower compared to coenzyme Q10-stearyl alcohol. This showed that coenzyme Q10 has a higher affinity for tristearin compared to stearyl alcohol because the former is more lipophilic than the latter [9]. The negative ΔG values indicate that the interactions occur spontaneously [37, 41].

The 3D visualization of molecular docking using DSV showed a hydrophobic bond between coenzyme Q10 with the lipids as shown in Figure 5. Intermolecular interactions include ionic, ion-dipole, and dipole-dipole, hydrogen, hydrophobic, and van der Waals bonds [42]. Furthermore, the van der Waals interactions tend to occur in nonpolar molecules [37]. It is an attractive force, between uncharged molecules or atoms, closely located at a distance of ±4–6 Å. The van der Waals forces are weak interaction which occurs due to the polarity of the induced atoms. However, in large amounts, it produces significant interaction and binding energy between molecules [42]. Coenzyme Q10, tristearin, and stearyl alcohol are nonpolar compounds [9]. The 3D visualization for the van der Waals interactions of coenzyme Q10-lipids was performed using ADT.

The skin penetration depth for (F1) and (F3) was not significantly different after 6 h, however, the fluorescence intensity of (F1) was higher than (F3), as shown in Figure 6. Furthermore, based on the particle size, (F1) was smaller compared to (F3). The EE and drug loading of coenzyme Q10 NLC (F1) were higher compared to (F3). Meanwhile, the skin penetration of NLC was affected by particle size and drug loading, this is supported by a previous study [43].

Conclusions

The coenzyme Q10 NLCs was developed using tristearin and stearyl alcohol, as well as IPP as solid and liquid lipids respectively. It possessed suitable physicochemical characteristics for dermal delivery and successfully penetrated the skin.

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Competing interests: The authors state no conflict of interest.

Informed consent: Not applicable.

Ethical approval: The experimental animals were carried out with the permission of the Animal Care and Use Committee (ACUC), Airlangga University (ethical clearance No. 2.KE.174.09.2019).

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The Journal of Basic and Clinical Physiology and Pharmacology (*JBCPP*) is a peer-reviewed bi-monthly published journal in experimental medicine. *JBCPP* publishes novel research in the physiological and pharmacological sciences, including Emergency Medicine, Oncology, Hematology and Coagulation disorders, Vascular Medicine, Gastroenterology, Liver Disease, Neurology and Cerebrovascular Diseases, Gender Medicine, Endocrinology, Diabetology and Metabolism, Cardiovascular Diseases, Heart Failure, Respiratory Disease, Geriatrics, Immunology and Rheumatology.

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