The effect of peppermint oil addition on the physical stability, irritability, and penetration of nanostructured lipid carrier coenzyme Q10

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Abstract

Background: Coenzyme Q10 is formulated into Nanostructured Lipid Carrier (NLC) added with peppermint oil (PO) 0% (F1), 1% (F2), 1.5% (F3) and 2% (F4) to increase its penetration.

Objective: This study aims to determine the effect of PO addition on the irritability, stability, and penetration of Coenzyme Q10 in the NLC.

Methods: Coenzyme Q10 NLC was prepared using the High Shear Homogenization method. Furthermore, physical characterization was carried out. Physical stability testing was carried out for 90 days at a temperature of 25±5°C and an RH of 60±10%. The *in vivo* irritation test was observed for mice's back skin after 24 hours while the penetration study was further evaluated at 2 hours of the sample application.

Results: Increasing the PO amount into Coenzyme Q10 NLC reduced the viscosity which was 329.1±15.5 cps for PO 0% to 219.9±2.9 cps for 2% addition. The observation of particle morphology showed that all NLC Coenzyme Q10 has a spherical particle shape with particle size between 188.25±13.22 to 197.80±14.19 nm. All formulas had high entrapment efficiency (>80%). PO addition did not cause changes in physical characteristics during 90 days of storage. The 24 hours' irritation test showed that F2 and F3 are non-irritating. By PO addition skin penetration improved at 2 hours' penetration study.

Conclusion: PO addition up to 2% reduced viscosity, but did not affect particle size and morphology of Coenzyme Q10 NLC. Addition of PO up to 1.5% increased entrapment efficiency, did not irritate and increased the penetration of Coenzyme Q10 NLC.

Introduction

Coenzyme Q10 (CoQ10) or ubiquinone is present in the inner mitochondrial membrane of body cells. It has a role in the electron transport chain for ATP synthesis, which is the primary source of cell energy and prevents lipid peroxidation.[1] This process can prevent the breakdown of collagen and elastin and avoid wrinkles.[2] However, the amount of CoQ10 in skin cells is so low that it needs to be supplemented from the antiaging cosmetic. The use of CoQ10 in cosmetics has several drawbacks, including low water solubility, large molecular weight, and high lipophilicity.[3] This causes CoQ10 to be retained in the stratum corneum, resulting in low skin penetration.

Nanostructured lipid carriers (NLC) are one of the delivery systems developed to increase the penetration of active compounds

into the skin.[4-6] NLC is a lipid nanoparticle generally consisting of solid lipids, liquid lipids, and emulsifiers having a particle size distribution of up to 500 nm.[7,8] The type and ratio of lipids used in NLC affect the morphology and particle size, trapping effectiveness, system stability, and penetration of active compounds into the skin. The combination of solid lipids and liquid lipids is known to affect the crystallinity index of the NLC matrix. Liquid lipids, plant oils, or fixed oils such as olive oil and VCO can be used.[6-9] The presence of plant oil liquid lipids in NLC can provide an emollient effect and increase occlusion by preventing water evaporation from the skin layer, resulting in increased skin hydration and increased penetration of active ingredients into the skin.[10] However, the presence of large amounts of plant oil causes a "greasy" feeling in use, so it is necessary to combine it with essential oils, for example, peppermint oil which also functions as an enhancer. Essential oils are volatile oils derived from aromatic plants that are not "oily" and do not have an emollient effect like fixed oil.

In this study, the effect of adding essential oil, namely peppermint oil (PO), was determined on the irritability, physical stability, and the penetration of CoQ10 from NCL-CoQ10, using a combination matrix of beeswax-cacao oleum and VCO.

Materials and Methods

Materials

It is not stated that the material used has a pharmaceutical grade. Coenzyme Q10 was purchased from Kangcare Bioindustry Co., Ltd., Nanjing, China. The cacao oleum was bought from Coffee and Cocoa Research Institute, Jember, Indonesia. The beeswax was a product of PT Brataco, Bekasi, Indonesia. The virgin coconut oil was purchased from CV Cocos Coconut, Surabaya, Indonesia. The peppermint oil was a product of Lansida Herbal Technology, Yogyakarta, Indonesia. Tween 80 and Span 80 were products of Sigma-Aldrich Co., LLC. (St. Louis, USA). The propylene glycol used in this study was a product of Dow Chemical Pacific, Singapore. Nipaguard EHP was a product of Clariant Produkte Deutschland GmbH, Burgkirchen, Germany. Ethanol 96% pro analysis, $NaH_2PO_4.2H_2O$ pro analysis, and $Na_2HPO_4.2H_2O$ pro analysis were purchased from Merck KGaA (Darmstadt, Germany).

The experimental animals

The experimental animals used were male mice (*Mus musculus*) aged 4-5 weeks, weighing 30-40 grams, healthy, and not injured. The number of experimental animals for the irritation test was three groups; each group consisted of 9 mice. There are four groups for the penetration test; each group consists of 6 mice. The procedure for animal experiment was approved by the ethics committee of the Faculty of Veterinary, Airlangga University, with a certificate of Ethical Clearance No. 2.KE.031.03.2021.

Preparation of the NLC-CoQ10-PO

The NLC Coenzyme Q10-PO system was made using the High Shear Homogenization method.[6] This method is relatively easy, low cost, the manufacturing process is fast, and it is possible to obtain nano-sized particles (~200 nm) for a lab-scale production.[10]

At first, beeswax is melted at a temperature of 65°C and heated in a mixture of Tween 80 and Span 80 at a temperature of 65°C. An aqueous phase mixture consisting of Nipaguard, propylene glycol, and phosphate buffer is heated in another container at the same temperature. After the beeswax is completely melted, cacao oleum is added to the melted beeswax. After the cacao oleum melts, a mixture of VCO, Coenzyme Q10, and PO is added to the melted Beeswax-cacao oleum. The next stage is mixing the water phase into the oil phase slowly. It is stirred with Ultra-Turrax® at 5,000 rpm for 10 minutes. After the aqueous phase was mixed entirely with the oil phase, the stirring speed was increased to 16,000 rpm for 2 minutes. Then cooling with a magnetic stirrer at a speed of 500 rpm to room temperature with aluminum foil covered until the NLC Coenzyme Q10-PO system is formed.

Physical characteristics evaluation

Physical characteristics evaluation includes; viscosity using a Cone and Plate viscometer, particle size using the Delsa™ Nano Submicron Particle Size Analyzer with the Dynamic Light Scattering method, particle morphology using a Transmission Electron Microscope (TEM). The examination results and measurements were then analyzed descriptively and statistically using the one-way ANOVA method.

Entrapment efficiency test

The maximum wavelength of Coenzyme Q10 in ethanol was determined for the standard working solution of Coenzyme Q10 at two concentrations, namely at 30 ppm and 40 ppm. The maximum wavelength of Coenzyme Q10 in ethanol is 274 nm. The NLC matrix produced absorption at the maximum wavelength of Coenzyme Q10, so in subsequent observations, absorbance measurements were carried out at three wavelengths, namely 264, 274, and 284 nm. Furthermore, the measurement of the entrapment efficiency percentage was carried out using the modified reported method.[9] A total of 100 mg of NLC Coenzyme Q10 was diluted in 10 mL of ethanol and then centrifuged at 1,500 rpm for 15 minutes, resulting in a separation between NLC and Coenzyme Q10. Coenzyme Q10, which is not entrapped in NLC, will be dispersed as supernatant. The supernatant obtained was filtered with MF-Millipore® Membrane Filter 0.45 µm, and absorbance was measured using a UV spectrophotometer. The entrapment efficiency is calculated using the equation formula

$$\text{Entrapment Efficiency (\%)} = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100$$

$W_{\text{initial drug}}$: total drug weight in NLC
$W_{\text{free drug}}$: free drug weight detected in the supernatant

Physical stability test

For the physical stability test, samples were stored for 90 days at a temperature of 25±5°C and an RH value of 60±10%. Then it was observed whether there were changes in color, odor, consistency, and separation, as well as checking the pH value, particle size, and Polydispersity Index (PI).

Irritation test

In vivo irritation test was carried out on the formulas of NLC Coenzyme Q10-PO (1%) and NLC Coenzyme Q10-PO (1.5%). The sample was applied to the skin on the back of the mice and then observed after 24 hours of application. After 24 hours of treatment, the mice were sacrificed, the back skin was excised, and histological preparations were made with hematoxylin-eosin staining. Further observations were evaluated using a Nikon E100 light microscope equipped with an Image Raster processing software.

Penetration test

The NLC Coenzyme Q10-PO penetration test was carried out *in vivo* on the back skin of mice. For the penetration test, the sample was given Nile Red indicator and then applied to the back of the mice after the hair was shaved. After 2 hours of the sample application, mice were sacrificed, and the treated skin was prepared for histological preparations. The depth of penetration was observed with a fluorescence microscope.

Results

Physical characteristics

The observations of physical characteristics include viscosity, particle size, particle morphology, and entrapment efficiency. The data obtained were then analyzed descriptively and statistically using the one-way ANOVA method.

Viscosity

The results of measuring the viscosity of the NLC Coenzyme Q10-PO system using a cone and plate viscometer, the viscosity values of NLC Coenzyme Q10 without the addition of PO (Figure 1 – F1), with the addition of PO 1% (Figure 1 – F2), 1.5% (Figure 1 – F3) and 2% (Figure 1 – F4) can be seen in Table 1 are 329.1±15.5, 307.6±35.9, 220.4±5.3 and 219.9±2.9 cps, respectively. Based on the one-way ANOVA statistical analysis result, the significant figure value (0.000) is smaller than 0.05, so it can be concluded that there is a significant difference in the viscosity value between the formulas. The Post Hoc Tamhane T2 analysis results showed a significant difference between F1, F3, and F4 (F1=F2 > F3=F4).

Particle size

The results of the NLC Coenzyme Q10-PO particle size examination using the dynamic light scattering method using the Delsa™ Nano Submicron Particle Size Analyzer, the particle sizes of F1, F2, F3, and F4 that can be seen in Table 1 were 188.25±13.22, 197.80±14.19, 190.90±9.47 and 187.50±8.71 nm, respectively.

Based on the results of statistical analysis of the particle size of NLC Coenzyme Q10-PO using the one-way ANOVA method, a significant figure value is 0.703 greater than 0.05, so it can be concluded that there is no significant difference in particle size between the formulas.

Particle morphology

The results of the morphology examination of the NLC Coenzyme Q10-PO particles using the TEM can be seen in Figure 1. All of the NLC Coenzyme Q10 had a spherical particle shape.

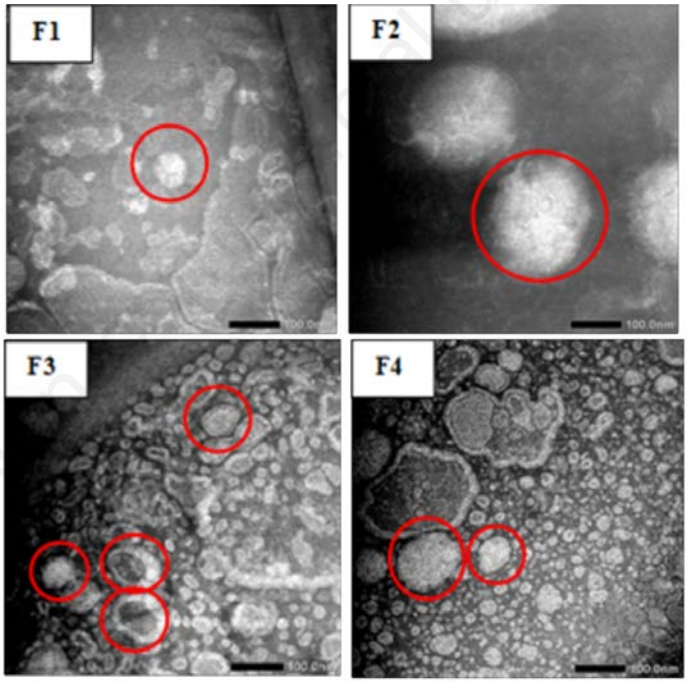

Figure 1. Morphology particles of NLC coenzyme Q10 without the addition of PO (F1); with the addition of 1% PO (F2), 1.5% (F3), and 2% PO (F4) using a transmission electron microscope (TEM) at 40,000x magnification.

Table 1. The results of determining the characteristics of NLC coenzyme Q10.

Formula	Viscosity (cps)	Particle size (nm)	EE (%)
F1	329.1±15.5	188.25±13.22	84.37±0.36
F2	307.6±35.9	197.80±14.19	85.83±0.21*
F3	220.4±5.3*	190.90±9.47	86.83±0.18*
F4	219.9±2.9*	187.50±8.71	85.39±0.31

*There are significant differences with F1.

Entrapment efficiency

Calculating the percentage of entrapment efficiency (EE) for the Coenzyme Q10 NLC system using the standard curve linear regression equation, y = 0.0128x+0.0057 with the r = 0.9996. The EE values of F1, F2, F3, and F4 that can be seen in Table 1 are 84.37±0.36, 85.83±0.21, 86.83±0.18 and 85.39±0.31%, respectively.

The data shows that the entrapment efficiency in all formulas is high (>80%). High entrapment efficiency is one of the advantages of NLC over other delivery systems, such as SLN.[11]

Based on the one-way ANOVA statistical analysis results, the significant figure value = 0.000 is smaller than 0.05, so it can be concluded that there is a significant difference in trapping efficiency between formulas, which F1 = F4 < F2 = F3.

Physical stability

It is known that all the visual color of formulas did not change after 90 days of storage as shown in Table 2. However, white spots started to appear on the surface of NLC Coenzyme Q10-PO 1% (F2) after the 14th day of storage and NLC Coenzyme Q10-PO 1.5% (F3) after the 30th day of storage. There was an increase in viscosity at F1, F2, and F3 during storage days of 30, 60, and 60, respectively. While in F4, the consistency did not change.

The pH value of the system during storage

The results of pH values measurement on the storage day of 1, 7, 14, 30, 60, and 90 for F1 were 5.914±0.003, 5.990±0.020, 6.016±0.006, 5.990±0.004, 6.009±0.012 and 6.038±0.013, for F2 were 5.898±0.001, 5.930±0.008, 6.065±0.039, 5.953±0.009, 6.053±0.011 and 6.047±0.005, respectively, for F3 were 5.906±0.004, 5.957±0.003, 6.046±0.029, 6.008±0.012, 6.011±0.013 and 6.070±0.011, for F4 were 5.904±0.008, 5.936±0.006, 6.084±0.026, 6.011±0.004, 6.040±0.015 and 6.095±0.002, respectively. There was a significant difference in pH value during 90 days of storage. The Post Hoc Tukey HSD analysis showed an increase in NLC Coenzyme Q10 on storage but was still within the specified pH value range of 6.0±0.5.

The particle size and polydispersity index of the system during storage

Stability observations of particle size and PI were carried out on the 14th and 90th day of storage. Particle size measurement results of F1, F2, F3, and F4 on the 14th day of storage were 171.0±7.9, 151.2±3.8, 164.0±6.4 and 150.5±4.9 nm, respectively. The particle size measurement results of F1, F2, F3, and F4 on the 90th day of storage were 164.7±6.7, 147.1±4.1, 157.7±11.2 and 169.7±17.4 nm, respectively. Statistically, there was no significant difference in the particle size of NLC Coenzyme Q10 during 90 days of storage. The particle size of the system was stable for 90 days of storage.

The results of the measurement of the Polydispersity Index F1, F2, F3, and F4 on the 14th day of storage were 0.222±0.036, 0.228±0.061, 0.180±0.003 and 0.274±0.033, respectively. The measurement results on the 90th day of storage were 0.236±0.047, 0.296±0.006, 0.260±0.069 and 0.246±0.123, respectively. These results showed no significant difference in the polydispersity index during 90 days of storage. The value of PI ≤0.3 indicated that the particle size of all NLC Coenzyme Q10 systems was homogeneous.

Irritation test results

Irritation test was performed on the selected formulas: NLC Coenzyme Q10-PO 1% (F2) and NLC Coenzyme Q10-PO 1.5% (F3), which had higher entrapment efficiency compared with NLC

Table 2. The physical stability test results of NLC coenzyme Q10 with various PO concentrations for 90 days' of storage.

Test group	R	FV	Test parameter					Mean FV	Mean R	Irritation score ± SD
			EL	SE	CFS	ICE	DA			
F1	1	1	0	1	1	1	0	0,6	0,40	0,22±0,20
(NLC CoQ10- PO 0%)		2	0	0	2	1	0	0,6		
		3	0	0	0	0	0	0		
	2	1	0	1	1	1	0	0,6	0,27	
		2	0	0	0	1	0	0,2		
		3	0	0	0	0	0	0		
	3	1	0	0	0	0	0	0	0	
		2	0	0	0	0	0	0		
		3	0	0	0	0	0	0		
F2	1	1	0	1	1	2	0	0,8	0,67	0,60±0,12
(NLC CoQ10-PO 1%)		2	0	0	1	2	0	0,6		
		3	0	0	1	2	0	0,6		
	2	1	0	0	1	2	0	0,6	0,67	
		2	0	0	1	2	0	0,6		
		3	0	0	2	2	0	0,8		
	3	1	0	0	1	2	0	0,6	0,47	
		2	0	0	0	2	0	0,4		
		3	0	0	1	1	0	0,4		
F3	1	1	0	1	1	1	0	0,6	0,60	0,45±0,17
(NLC CoQ10-PO 1.5%)		2	0	2	1	1	0	0,8		
		3	0	1	1	0	0	0,4		
	2	1	0	0	2	1	0	0,6	0,47	
		2	0	0	2	0	0	0,4		
		3	0	0	2	0	0	0,4		
	3	1	0	1	0	0	0	0,2	0,27	
		2	0	0	0	0	0	0		
		3	0	0	3	0	0	0,6		

R = Replicate; FV = Field of View; EL = Epidermis Liquefaction; SE = Subepidermis Edema; CFS = Collagen Fiber Swelling; ICI = Inflammatory Cell Infiltration; DA = Degeneration Appendages. Score 0 = no changes; 1 = very light; 2 = light; 3 = medium; 4 = severe

Coenzyme Q10 without the addition of PO. The results of microscopic histopathological observations can be seen in Figure 2.

Evaluation was done by scoring technique. The results of the histopathological scoring of the irritation test can be seen in Table 3. There was no significant difference in the irritation score between the formulas. Coenzyme Q10 NLC, with the addition of PO up to 1.5%, had a low average irritation score (0.22±0.20), where the score indicated almost no irritation after 24 hours of system application. NLC Coenzyme Q10-PO 1% (F2) and NLC Coenzyme Q10-PO 1.5% (F3) had irritation scores that were not significantly different from NLC Coenzyme Q10-PO 0% (F1).

The irritation test results showed that adding PO at a concentration of 1% and 1.5% into NLC Coenzyme Q10 systems was not irritating to the skin because it had an average irritation score of <1.

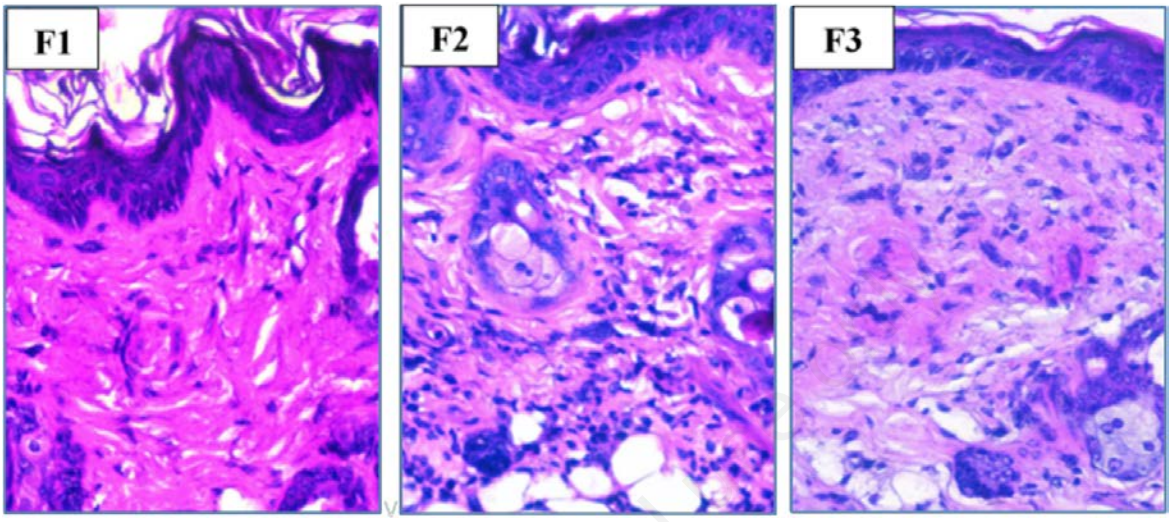

Figure 2. Microscopic observation of the back skin of mice after 24 hours of application of NLC coenzyme Q10-PO 0% (F1), NLC coenzyme Q10-PO 1% (F2), NLC coenzyme Q10-PO 1.5% (F3) using Nikon E100 light microscope at 400x magnification.

Table 3. Scoring results of irritation test of coenzyme Q10-PO system on mice back skin after 24 hours of application.

Test group	R	FV	Test parameter					Mean FV	Mean R	Irritation score ± SD
			EL	SE	CFS	ICE	DA			
F1	1	1	0	1	1	1	0	0,6	0,40	0,22±0,20
(NLC CoQ10- PO 0%)		2	0	0	2	1	0	0,6		
		3	0	0	0	0	0	0		
	2	1	0	1	1	1	0	0,6	0,27	
		2	0	0	0	1	0	0,2		
		3	0	0	0	0	0	0		
	3	1	0	0	0	0	0	0	0	
		2	0	0	0	0	0	0		
		3	0	0	0	0	0	0		
F2	1	1	0	1	1	2	0	0,8	0,67	0,60±0,12
(NLC CoQ10-PO 1%)		2	0	0	1	2	0	0,6		
		3	0	0	1	2	0	0,6		
	2	1	0	0	1	2	0	0,6	0,67	
		2	0	0	1	2	0	0,6		
		3	0	0	2	2	0	0,8		
	3	1	0	0	1	2	0	0,6	0,47	
		2	0	0	0	2	0	0,4		
		3	0	0	1	1	0	0,4		
F3	1	1	0	1	1	1	0	0,6	0,60	0,45±0,17
(NLC CoQ10-PO 1.5%)		2	0	2	1	1	0	0,8		
		3	0	1	1	0	0	0,4		
	2	1	0	0	2	1	0	0,6	0,47	
		2	0	0	2	0	0	0,4		
		3	0	0	2	0	0	0,4		
	3	1	0	1	0	0	0	0,2	0,27	
		2	0	0	0	0	0	0		
		3	0	0	3	0	0	0,6		

R = Replicate; FV = Field of View; EL = Epidermis Liquefaction; SE = Subepidermis Edema; CFS = Collagen Fiber Swelling; ICI = Inflammatory Cell Infiltration; DA = Degeneration Appendages Score 0 = no changes; 1 = very light; 2 = light; 3 = medium; 4 = severe.

Penetration test results

The results of the penetration test of NLC Coenzyme Q10 systems of F1, F2, F3 on the back skin of mice compare with mice without treatment as a negative control for 2 hours, with a microscopic Nile Red indicator, can be seen in Figure 3.

Figure 3 shows that the control without the Nile Red indicator did not show any fluorescence in the epidermis, dermis, or hypodermis layers. In the Nile Red solution, VCO fluorescence was seen in the epidermis (+), dermis (++), and hypodermis (++). In NLC, Coenzyme Q10 (F1) fluorescence was seen in the epidermis (++), dermis (+), and hypodermis (+). In NLC Coenzyme Q10-PO, 1% (F2) fluorescence was seen in the epidermis (+), dermis (++), and hypodermis (+++). In NLC Coenzyme Q10-PO, 1.5% (F3) fluorescence was seen in the epidermis (+), dermis (+), and hypodermis (++).

Discussion

Based on viscosity data, the decrease in viscosity occurred after the addition of PO probably due to a liquefying effect of PO on solid lipids because adding PO in a fairly low concentration causes the system's viscosity to decrease significantly. The liquefying effect is thought to come from the interaction of the largest PO content, menthol, with the fatty acid components in beeswax and cacao oleum. The interaction of menthol with these components in specific ratios can form a eutectic mixture with a much lower melting point.[12-14] It is known that a mixture of menthol with stearic acid at a certain ratio causes the melting point of stearic acid to decrease from 73°C to 20-40°C.[13] The mixture of menthol with palmitic acid in a ratio of 12:1 causes the melting point of palmitic acid to decrease from 62.9°C to 23.16°C.[14] This decrease in melting point is thought to impact the decrease in system viscosity.

The particle size of NLC Coenzyme Q10 with the addition of PO up to 2% produced in this study was not significantly different. Based on Agustin's research (2020), the addition of liquid lipids into the system will reduce the viscosity so that the dispersion energy needed by the system to break down the particle size becomes lower, resulting in a smaller particle size.[15] In this study, adding 1.5% and 2.0% PO decreased the viscosity of NLC Coenzyme Q10 but did not reduce the particle size. This is probably due to the decrease in viscosity that has yet to make the dispersion energy decrease to break down the particle size into smaller ones.

The results of the preparation of NLC Coenzyme Q10 with adding PO up to 2% produced spherical particles. Spherical particles can avoid interparticle aggregation because they have less contact and friction. The spherical particles also can help penetrate the active ingredients into the skin better.[16] In Figure 1, it can be seen that there were many particles with irregular shapes, maybe Coenzyme Q10, which is not trapped in the NLC and undergoes recrystallization, or substances present in the aqueous phase, such as buffers and preservatives.

From the entrapment efficiency data, an increase in F2 (85.83%) and F3 (86.83%) was observed probably because the addition of PO into the system caused a higher partition of Coenzyme Q10 in the lipid matrix.[17] The high concentration of liquid lipids increases the drug trapped in the NLC because the liquid lipid can dissolve more drug molecules.[18] However, the maximal drug entrapment efficiency in the lipid matrix also depends on the optimal ratio of solid lipids to liquid lipids.[19] From the data obtained, 1.5% PO concentration is the optimal concentration that can increase the entrapment efficiency of Coenzyme Q10 into the NLC matrix.

There was an increase in viscosity, and white spots appeared at F2 and F3 during storage day of 30 and 60, while in F4, the consistency did not change. This is thought to be due to a phenomenon often called "fat bloom", caused by using cacao oleum in the formula. The visible fat blooms on the surface result from the migration of solid lipids (white spots), often with the transformation of cacao oleum from β-V to β-VI polymorphs.[20] Fat blooms are mainly triggered by environmental conditions, such as temperature fluctuations, storage temperatures higher than the melting point of β-V polymorphs, and poor tempering processes during manufacture so that crystal nuclei do not form. This condition becomes even more complicated when other fats or oils other than cacao oleum are present in the system.[21] Fat bloom is an aesthetic problem and does not affect product quality. It can be minimized by adding lecithin to the base and lowering the melting temperature during preparation.[22,23] Based on Thuy *et al*. (2020), Tween 80 surfactant can pre-

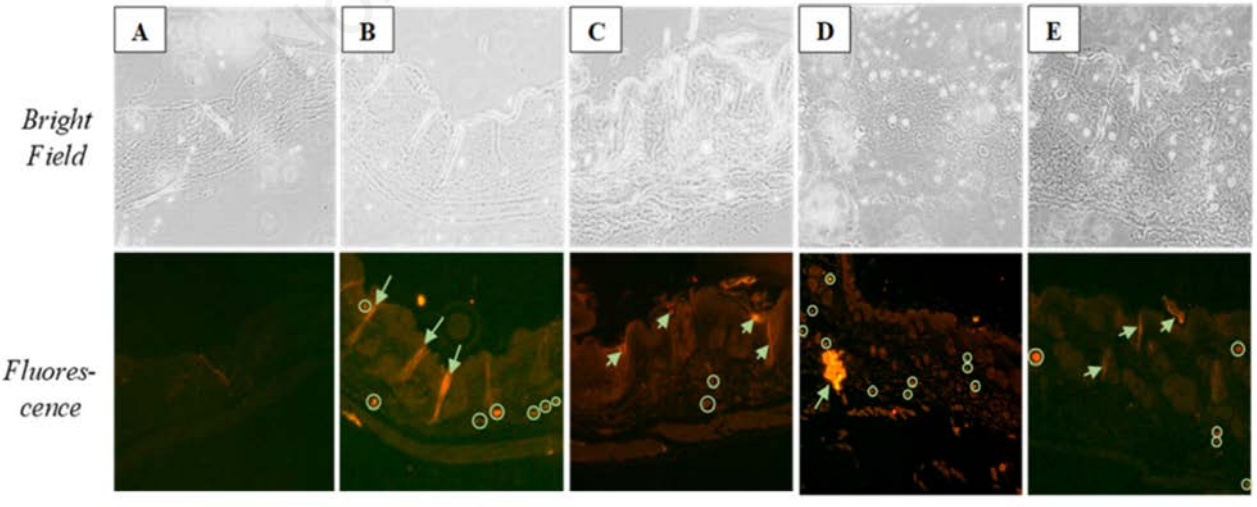

Figure 3. Penetration test results on microscopic observation of the back skin histological preparations of male mice after 2 hours of application; control without treatment (A), 0.007% Nile Red solution in VCO (B), NLC coenzyme Q10 (C), NLC coenzyme Q10-PO 1 %) (D) and NLC coenzyme Q10-PO 1.5% (E) using a fluorescent microscope at 200x magnification

vent fat bloom. In the formula of NLC Coenzyme Q10-PO, there are Tween 80 and Span 80, but they have been unable to prevent fat bloom, probably because the number is still lacking.[24]

All formulas have the initial consistency of a thick liquid. Changes in the consistency of F1, F2, and F3 to become more viscous from the 30th to the 90th day of storage could occur due to the solidification of lipids during storage below the melting temperature of the lipids. Storage was carried out at 25±5°C and an RH of 60±10%. The solid lipid used is beeswax, which has a melting temperature of 62–65°C, and cacao oleum has a melting temperature of 31-34°C. In contrast, the liquid lipid used is VCO, which has a melting temperature of 23-26°C.[25] However, changes in the consistency of the system are reversible. When the NLC is at a higher room temperature, the viscosity returns to its previous state. In F4, there was no significant change in consistency, presumably due to the high liquid lipid concentration for the system to maintain its consistency.

Based on the data in Table 2, it can be seen that there is a slight increase in the irritation score due to the addition of PO but it is still <1 (nonirritant). PO is known to cause irritation and skin reactions immediately after contact or contact dermatitis in the form of urticarial.[26,27] The compound strongly suspected to be the cause is menthol, the largest content of PO.[28] In addition, the menthol content in PO stimulates nociceptors which regularly release vasodilator peptides, resulting in a burning sensation on the skin.[29] In this study, addition PO up to 1.5% was known to not be able to irritate.

The penetration test carried out on F2 (NLC Coenzyme Q10-PO 1%) has the advantage of the smallest polydispersity index among the three formulas, and the entrapment efficiency is relatively high. In contrast, F2 (NLC Coenzyme Q10-PO 1.5%) has the advantage of the highest trapping efficiency. The penetration test results showed that NLC Coenzyme Q10-PO 1% (F2) was mainly in the hypodermis layer, and NLC Coenzyme Q10-PO was 1.5% (F3) less in both the dermis and the hypodermis. This is presumably due to the small particle size and the addition of PO, which can increase penetration. In general, the ideal particle size of NLC for dermal targeting is <500 nm because the skin pore size ranges from 250 – 500 nm.[30,31] The particle size of the NLC system in this study was <200 nm. Small particle sizes tend to enter the deepest part of the skin, while larger particle sizes mainly accumulate in the epidermis and dermis.[32] It is suspected that 2 hours after application, the NLC Coenzyme Q10 system has passed through the dermis and reached the hypodermis, especially at the addition of 1.5% PO, which is suspected of having entered the deepest layer of NLC particles, and started leaving the skin. In cosmetic products for dermal purposes, certain penetration into the skin is desired, especially when the active ingredient must be localized in the skin but not absorbed systemically.[33] The dermis is the target of penetration of the Coenzyme Q10 NLC system as an antiaging system.[34] Irritation and penetration tests were not carried out on F4 because there was no increase in EE.

Conclusions

The addition of PO up to 2% affected the physical characteristics of the Coenzyme Q10 NLC system, namely reducing viscosity but did not affect particle size and morphology. The addition of PO up to 1.5% increased entrapment efficiency, did not irritate and increased the penetration of NLC Coenzyme Q10.

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