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Effect of Peppermint Oil on the Characteristics and Physical Stability of Nanostructured Lipid Carrier-CoQ10

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ABSTRACT

Peppermint oil (PO) is one of the essential oils used as a enhancer in nanostructured lipid carriers (NLC), which affects the characteristics and stability of NLC. This study was aimed at determining the effect of PO on the characteristics and stability of NLC-coenzyme Q10 (NLC-CoQ10). NLC containing 1% CoQ10 was added to various concentrations (0, 1, 1.5, and 2%) of PO, namely F1-F4, respectively. The characteristics (odour, shape, viscosity, pH, particle size, and polydispersity index) of F1, F2, F3, and F4 were determined. Physical stability, centrifugation, and thermal cycle tests were performed. The results showed that adding 1-2% PO caused NLC-CoQ10 to have a distinctive PO odour, a spherical shape, and viscosity values of 329.1±10.5, 307.6±27.4, 220.4±4.0, and 219.9±2.2 cps for F1, F2, F3, and F4, respectively. The pH values ranged from 6.33 to 6.36, while the particle sizes were 188.25±13.22, 197.80±14.19, 190.90±9.47, and 187.50±8.71 nm, respectively. The polydispersity index was below 0.3; and the zeta potential values ranged from –44.30 mV to –52.74 mV. F1, F2, and F3 remained stable after 30 days, whereas F4 became more fluid. The formulas performed well in a centrifuge test. The F2, F3, and F4 thermal cycle tests were all stable, but the F1 separated in the first cycle. The addition of 1, 1.5, and 2% PO did not affect the spherical morphology, the pH value, particle size, or the polydispersity index. The addition of 1.5 and 2% PO reduced viscosity, while the addition of 1 and 1.5% reduced zeta potential but increased stability.

Keywords: Nanostructured lipid carrier (NLC), CoQ10, Peppermint oil, Characteristics, Physical Stability.

Introduction

Ageing is a multi-system degenerative process that occurs in all organs of the body, including the skin. It can be categorized into intrinsic and extrinsic aging. Intrinsic aging is also known as the natural aging process, which is a continuous process that is in line with increasing age.[1] Meanwhile, extrinsic aging is influenced by factors that come from outside, such as smoking, nutritional deficiencies, and excessive sun exposure. Sun exposure can cause 80% of facial aging.[2] Exposure to sunlight or UV rays can initiate the formation of reactive oxygen species (ROS).[3] The presence of ROS can cause damage to the collagen connective tissues and reduce the size of fibroblasts. This increases MMPs and CCN1 while lowering TGF signaling, resulting in increased collagen fiber damage and inflammation as well as decreased collagen synthesis.[3]

This problem can be overcome by controlling the number of ROS in the cells. One of the compounds that can control ROS in the body is Coenzyme Q10 (CoQ10).[4] CoQ10 is a fat-soluble compound that resides in the mitochondria of cells in the body, which can act as an anti-aging.[5,6] The production of ROS in the body can be controlled and lipid peroxidation can be slowed or even prevented by CoQ10.[4] In addition, CoQ10 can also inhibit MMPs, one of which is collagenase, so it can prevent the breakdown of collagen tissue.[2]

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Endogenous CoQ10 production in the body can decrease with aging and environmental factors, so it is necessary to add CoQ10 externally.[7] However, the addition of CoQ10 externally limits penetration because it has high lipophilicity (Log P>10) and low water solubility.[6] To overcome this problem, a delivery system that can increase its penetration into the skin can be used, namely the nanostructure lipid carrier (NLC).[4] NLC consists of several mixtures of solid and liquid lipids or a combination of solid lipids and single liquid lipids that lead to the formation of amorphous solids.[4]

According to research, a combination of beeswax-oleum cacao (100:0, 50:50, 25:75, or 0:100) with liquid lipid *Aleuritis moluccana* seed (AMS) oil in a combined ratio of 25:75 has the best physical stability, as evidenced by the lack of phase separation after 8 weeks of storage.[8] Another study using virgin coconut oil (VCO) as a liquid lipid combined with solid lipid beeswax-oleum cacao (25:75) in NLC-APMS was conducted with a solid lipid: liquid lipid ratio of 60:40; 70:30; and 80:20, showing good results with a ratio of solid lipids to liquid lipids (60:40). These results are based on the particle size of 236.00±17.15 nm; the viscosity of 126.90±17.34 cPs, and an entrapment efficiency of 43.37±1.10%.[8] It is known that the entrapment efficiency of CoQ10 in the NLC system with solid lipids cetyl palmitate and olive oil liquid lipids are 74.33±0.87%.[9] However, the penetration of the NLC system is still lacking when compared to the penetration of nanoemulsion (NE), whereas a study of CoQ10 in NE at 6 hours had a penetration depth of 1258.53±7.03 µm.[9] Also in the NLC system, after 6 hours, can penetrate as deep as 639.34±17.69 µm.[9] Based on this, the NLC system needs an enhancer that can increase penetration. There are various types of enhancers, one of which is the natural penetration enhancer (NPE). This is an essential oil that interacts with tissue components to reduce the skin's resistance system so that it can penetrate the stratum corneum without damaging skin cells. D-limone and 1,8-cineole are known to modify permeation diffusion by disrupting the lipid layer in the stratum corneum.[10] One of

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the essential oils that can function as an enhancer is peppermint oil (PO). The addition of PO as a liquid lipid to the NLC system can affect the characteristics and stability. Therefore, this research was conducted to investigate the effect of various concentrations of PO on the characteristics and stability of NLC-CoQ10.

Materials and Methods

Materials used

The materials used in this study were beeswax, oleum cacao (Research Center for Coffee and Cocoa, Jember Indonesia), CoQ10, Span80 (Sigma Aldrich), Tween 80 (Sigma Aldrich), propylene glycol, Nipaguard, Na_2HPO_4 pro analysis (Merck), and NaH_2PO_4 pro analysis (Merck). Unless otherwise mentioned, the materials had a purity level of pharmaceutical grade.

NLC-CoQ10-PO preparation

The NLC matrix consisted of beeswax, oleum cacao, VCO with surfactant Tween 80, Span 80, and co-surfactant propylene glycol, and 1% CoQ10 (w/w) of the anti-aging active ingredient. Peppermint oil was prepared by high shear homogenization in four distinct concentrations: 0% (F1), 1% (F2), 1.5% (F3), and 2% (F4).

Evaluations of NLC-CoQ10-PO

Organoleptic, pH value, viscosity, particle size, polydispersity index (PDI), and zeta potential were some of the characteristics of NCL-CoQ10-PO, formulae F1, F2, F3, and F4 that were observed. The morphological characteristics of NLC-CoQ10-PO were carried out by transmission electron microscopy (TEM) type JEM-14000. The stability test included storage at 20 ± 1^{o}C and 65% relative humidity (RH) for 30 days, the thermal cycle method (3 cycles, each cycle consisting of 48 hours at 40°C followed by 48 hours at 2-8°C), and centrifugation at 3500 rpm speed for 3 x 15 minutes.

Statistical analysis

The statistical analysis was performed for viscosity value, pH value, particle size, PDI, and zeta potential with a one-way analysis of variance (ANOVA) followed by a post hoc Tukey HSD test using SPSS software version 22.0 (SPSS, USA). Statistically significant differences were defined as a p-value < 0.05. Data on morphology, odour, colour, viscosity, and separation were analyzed descriptively.

Results and Discussion

Organoleptic properties of NLC-CoQ10-PO

The results of visual organoleptic observation, which included colour, odour, and consistency, are presented in Table 1 and Figure 1. The addition of PO to NCL-CoQ10 caused a change in the odour that smelled like PO but did not change the colour or visual consistency. This may be because PO is a liquid lipid that has a fairly high viscosity, and relatively small amounts were added.

Morphological characteristics of NLC-CoQ10-PO

According to the results of the TEM type JEM-14000 morphological observation (Figure 2), the obtained NLC-CoQ10-PO morphology had a spherical shape or was close to spherical. The particle morphology relates to the physical stability of the system and the ease of penetration into the skin. More spherical particle morphology results in greater physical stability and increased penetration rate. Particles with spherical morphology have less contact with the media than non-spherical particles, so the risk of agglomeration between particles tends to be lower.

Effect of peppermint oil on the viscosity of NLC-CoQ10

The results of the NLC-CoQ10-PO viscosity measurement by cone and plate viscometer are shown in Table 2. Based on the statistical analysis using one-way ANOVA, there was a significant difference (P> 0.05) in viscosity between formulas. According to the post hoc Tukey HSD test, it was revealed that the viscosity of F1 = F2 > F3 = F4. Therefore, it can be inferred that the addition of 1.5 - 2% PO reduces the viscosity of NLC-CoQ10.

Table 1: Organoleptic characteristics of NLC-CoQ10-PO

Formula	Observation result		
	Colour	**Aroma**	**Consistency**
F1 (0% PO)	yellow	odourless	viscous liquid
F2 (1% PO)	yellow	peppermint oil smell	viscous liquid
F3 (1.5% PO)	yellow	peppermint oil smell	viscous liquid
F4 (2% PO)	yellow	peppermint oil smell	viscous liquid

Table 2: Viscosity measurement of NLC-CoQ10-PO

Formula	Replication of Viscosity Measurement (cps)			Mean Viscosity ± SD
	1	2	3	
F1 (0% PO)	344.3	313.4	329.6	329.1 ± 15.5
F2 (1% PO)	348.8	291.2	282.9	307.6 ± 35.9
F3 (1.5% PO)	226.4	218.6	216.2	220.4 ± 5.3
F4 (2% PO)	221.0	222.0	216.6	219.9 ± 2.9

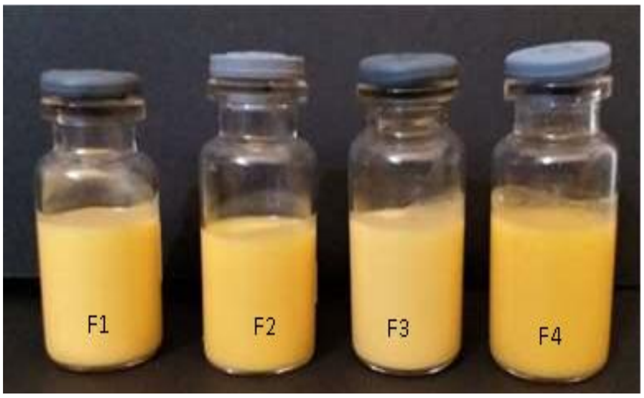

Figure 1: NLC-CoQ10-PO formula.

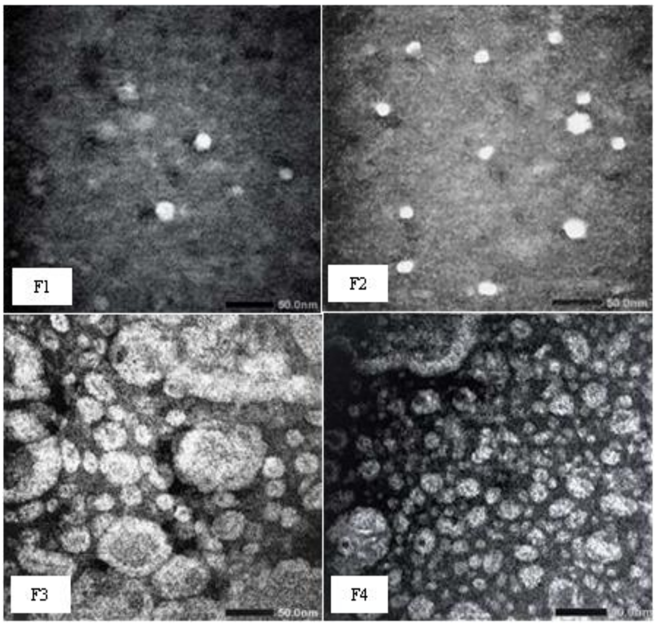

Figure 2: Morphology of the NLC-CoQ10 PO formula under the transmission electron microscope.
F1: 0% PO; F2: 1% PO; F3: 1.5% PO; F4: 2% PO; PO: Peppermint oil; TEM type JEM 14000 with marker scale of 50.0 nm.

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Effect of peppermint oil on the pH of NLC-CoQ10

Table 3 shows the outcome of pH measurement for NLC-CoQ10-PO; F1 (0% PO), F2 (1% PO), F3 (1.5% PO), and F4 (2% PO). The pH of NLC-CoQ10 was unaffected by the addition of PO. NLC-CoQ10-PO has a pH range of 6.33 to 6.36, which is similar to that of the skin. When the NLC-CoQ10-PO formula is administered to the skin within this pH range, it will not irritate.[8]

Effect of peppermint oil on the particle size of NLC-CoQ10

The results of the particle size measurement of NLC-CoQ10-PO; F1 (0% PO), F2 (1% PO), F3 (1.5% PO), and F4 (2% PO) using the Delsa ™ Nano Submicron Particle Size Analyzer are presented in Table 4. There were no significant differences (P> 0.05) in particle size between the formulas. This was due to only a slight increase in the PO concentration of each formula. Because the slight change in PO content between the formulas did not result in a significant difference (P> 0.05) in viscosity. The droplet size was not affected when the same power was used to disperse the system. Physical stability and effectiveness are influenced by particle size. Large particles produce unstable systems, according to Stokes' Law, which stipulates that the rate of sedimentation is directly proportional to particle size; larger particles settle faster. Meanwhile, the lipid delivery system's particle size of less than 300 nm will produce nanoparticles that can penetrate the dermis layer of the skin and deliver drugs. However, all NLC-CoQ10-PO formulas yielded particle sizes ≤ 300 nm, which is within the range of lipid nanoparticles that can penetrate and deliver drugs up to the skin layer.[11,12]

Polydispersity index measurement of NLC-CoQ10-PO

The outcome of the polydispersity index measurement of NLC-CoQ10-PO, formulas F1 (0% PO), F2 (1% PO), F3 (1.5% PO), and F4 (2% PO) are shown in Table 5. The polydispersity index of all NLC-CoQ10-PO ranges from 0.185 to 0.232 ≤ 0.3. A system with a polydispersity index value ≤ 0.3 indicates that the system has a homogeneous particle size and tends to be physically stable.[11,13]

Zeta potential measurement of NLC-CoQ10-PO

The zeta potential describes the electrical potential of the particles in the system, which can predict the probability of dispersion, aggregation, or flocculation.[13] According to the results of the zeta potential measurement of NLC-CoQ10-PO (Table 6), it was observed that the zeta potential value of all NLC-CoQ10-PO ranged from –44.30 to -52.74 mV. The system with a zeta potential value of between -30 to -60 mV is physically stable, with a low tendency for particle aggregation due to high electrical repulsion.[14] Because of the charge on the surface of the particles and the presence of electrolytes in the dispersion medium, the zeta potential value is formed. The charge on the surface of the NLC-CoQ10-PO particles can be produced by the presence of Tween 80 and Span 80 on the particle surface. Tween 80 and Span 80 are non-ionic surfactants that act as stabilizers in NLC systems by adsorbing anionic ions (OH^-) from the water and transporting them to the particle surface.[15] Lipid-based nanoparticles also generate a negative zeta potential because lipid molecules can absorb OH^- ions from water molecules.[16]

Physical stability of NLC-CoQ10-PO at a storage temperature of 20 ± 1°C and 65 % RH

Table 7 shows the results of a 30-day organoleptic analysis of NLC-CoQ10-PO at a temperature of 20 ± 1°C and a RH of 65%. The organoleptic observations of formulas F1, F2, and F3 after 30 days of storage were stable. However, the consistency of F4 becomes more fluid. When peppermint oil was added at 2%, the crystal lattice became too loose, allowing liquid lipids to escape from the NLC matrix.

Results of NLC-CoQ10-PO centrifugation and thermal cycle tests

The results of the NLC-CoQ10-PO centrifugation test (Table 8 and Figure 2) revealed that the addition of PO with a concentration of 1 to 2% did not affect the physical stability of NLC-CoQ10. There was no colour change or phase separation.

Table 3: pH measurement of NLC-CoQ10-PO

Formula	Replication of pH Measurement			Mean pH ± SD
	1	**2**	**3**	
F1 (0% PO)	6.33	6.34	6.34	6.34 ± 0.01
F2 (1% PO)	6.36	6.37	6.35	6.36 ± 0.01
F3 (1.5% PO)	6.30	6.30	6.30	6.30 ± 0.00
F4 (2% PO)	6.33	6.34	6.33	6.33 ± 0.01

Table 4: Particle size of NLC-CoQ10-PO

Formula	Replication	Particle Size (nm)	Mean Particle Size ± SD (nm)
F1 (0% PO)	1	178.90	188.25 ± 13.22
	2	197.60	
	3	188.27	
F2 (1% PO)	1	185.90	197.80 ± 14.19
	2	213.50	
	3	194.00	
F3 (1.5% PO)	1	200.70	190.90 ± 9.47
	2	181.80	
	3	190.20	
F4 (2% PO)	1	181.60	187.50 ± 8.71
	2	183.40	
	3	197.50	

Table 5: Polydispersity index of NLC-CoQ10-PO

Formula	Replication	PI	Mean PI ± SD
F1 (0% PO)	1	0.223	0.203 ± 0.03
	2	0.183	
	3	0.201	
F2 (1% PO)	1	0.206	0.185 ± 0.12
	2	0.164	
	3	0.185	
F3 (1.5% PO)	1	0.285	0.232 ± 0.05
	2	0.224	
	3	0.189	
F4 (2% PO)	1	0.296	0.219 ± 0.08
	2	0.138	
	3	0.222	

Table 6: Zeta potential measurement of NLC-CoQ10-PO

Formula	Mean Zeta potential ± SD (mV)
F1 (0% PO)	-52.74 ± 2.76
F2 (1% PO)	-44.30 ± 3.04
F3 (1.5% PO)	-45.26 ± 0.99
F4 (2% PO)	-50.96 ± 2.43

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Table 7: Organoleptic characteristics of NLC-CoQ10-PO at 20 ± 1°C and 65% relative humidity for 30 days

System	Day-	Observation Type		
		Colour	**Separation**	**Consistency**
F1 (0% PO)	1	Yellow	No	Viscous liquid
	7	Yellow	No	Viscous liquid
	14	Yellow	No	Viscous liquid
	30	Yellow	No	Viscous liquid
F2 (1% PO)	1	Yellow	No	Viscous liquid
	7	Yellow	No	Viscous liquid
	14	Yellow	No	Viscous liquid
	30	Yellow	No	Viscous liquid
F3 (1.5% PO)	1	Yellow	No	Viscous liquid
	7	Yellow	No	Viscous liquid
	14	Yellow	No	Viscous liquid
	30	Yellow	No	Viscous liquid
F4 (2% PO)	1	Yellow	No	Viscous liquid
	7	Yellow	No	More fluid
	14	Yellow	No	More fluid
	30	Yellow	No	More fluid

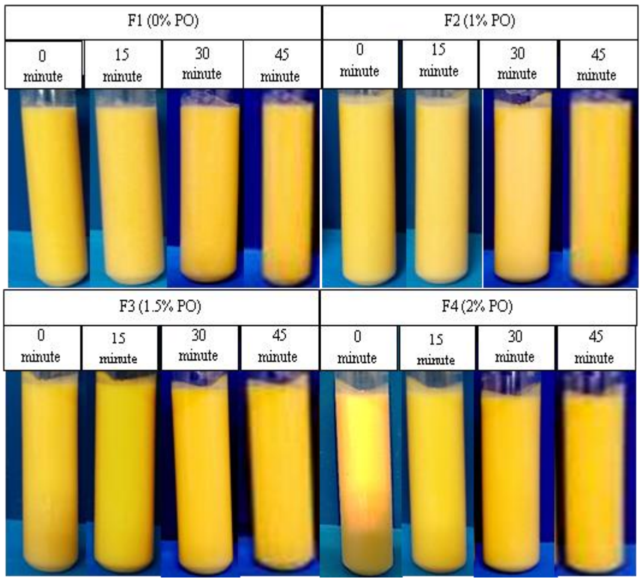

Figure 3: Centrifugation test result of NCL-CoQ10-PO formula at 3500 rpm, for 3 x 15 minutes.
F1: 0% PO; F2: 1% PO; F3: 1.5% PO; F4: 2% PO; PO: Peppermint oil

The thermal cycle was repeated three times, with each cycle consisting of sample storage at 40°C for 48 hours, followed by transfer to a temperature of 2-8°C for 48 hours. The F1 formula (0% PO) demonstrated separation from the first cycle (Table 9 and Figure 3). It was probably because the VCO content started to harden at a low temperature, which was less than 20°C.[17]
In a previous study, the results of the thermal cycle test for VCO emulsion preparations using Tween 80 and Span 80 surfactants showed that the viscosity, droplet size, and polydispersity index (PI) increased compared to earlier treatment.[18] According to Stokes law, the separation rate increases with increasing particle size. In addition, the increase in PI value after the thermal cycle test indicates that there is instability in the formulas.[19]

Table 8: Centrifugation test result of NLC CoQ10-PO at 3500 rpm, for 3 x 15 minutes

Formula	Time (minutes)	Colour	Separation
F1 (0% PO)	0	Yellow	No
	15	Yellow	No
	30	Yellow	No
	45	Yellow	No
F2 (1% PO)	0	Yellow	No
	15	Yellow	No
	30	Yellow	No
	45	Yellow	No
F3 (1.5% PO)	0	Yellow	No
	15	Yellow	No
	30	Yellow	No
	45	Yellow	No
F4 (2% PO)	0	Yellow	No
	15	Yellow	No
	30	Yellow	No
	45	Yellow	No

As a result, it is thought to cause a separation in the NLC-CoQ10 system. However, there was no separation in F2, F3, or F4. It is most likely because the addition of PO, which is a liquid lipid that does not solidify at cold temperatures, hinders the compaction of VCO.

Conclusion

The addition of 1, 1.5, and 2% PO to NLC-CoQ10 causes it to smell like PO, but it does not affect the spherical shape, pH value, particle size, or polydispersity index. The addition of 1.5 and 2% PO reduces

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viscosity, while the addition of 1 and 1.5% PO reduces zeta potential but increases its stability.

Conflict of Interest

Authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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