The Effect Of Solid Lipid Concentration on the Physical Stability of Nanostructured Lipid Carriers (NLC) Coenzyme Q10 Particles

Silvi Ayu Wulansari[1*], Umarudin[2], Rakhmi Hidayati[3]

[1-2]Akademi Farmasi Surabaya
[3]Institut Teknologi Kesehatan Cendekia Utama Kudus

*Corresponding Author: silviayu25@gmail.com

Abstract. Coenzyme Q10 is a natural antioxidant that is unstable and quickly degraded when exposed to light, so it is necessary to choose a delivery system that can improve the stability of coenzyme Q10, extend the adequate time, and deliver coenzyme Q10 to penetrate the stratum corneum and achieve defense. Coenzyme Q10 can be formulated in Nanostructured Lipid Carrier (NLC) preparations by combining solid lipids (Myristic acid), liquid lipids (Caprylic), which are stabilized by surfactants (Span 80 and Tween 80), and co-surfactants (Propylenglykol). This study aims to determine the effect of variations in the concentration of solid lipids (Myristic Acid) on the zeta potential of Coenzyme Q10 Nanostructured Lipid Carrier (NLC) preparations. The concentration of myristic acid used was F1, F2, and F3. Physical characteristics are evaluated 24 hours after the practice is complete. Observations include organoleptic, spreadability, pH, zeta potential, and droplet size. The research data were processed statistically using ANOVA One Way. The results showed that the myristic acid concentration affected zeta potential with a significantly different impact of 0,001 (p-value signifikan < 0,05). Still, the myristic acid concentration did not affect the particle size, with the result not considerably different at 0,966 (p-value signifikan > 0,05).

Key words: Coenzym Q10, Myristic acid, Nanostructured Lipid Carrier

INTRODUCTION

Coenzyme Q10 is a natural lipid-soluble antioxidant and exhibits intense antioxidant activity. Coenzyme Q10 works to inhibit lipid and protein peroxidation, has the potential to scavenge free radicals, and plays a central role in mitochondrial oxidative phosphorylation (Dewi et al., 2019). Coenzyme Q10 is unstable and quickly degrades when exposed to light, so it is necessary to choose a delivery system that can improve the stability of coenzyme Q10, extend the adequate time, and deliver coenzyme Q10 to penetrate the stratum corneum and achieve controlled release (Patimah et al., 2020). One delivery that can be used. Namely, Coenzyme Q10 can be formulated in a Nanostructured Lipid Carrier (NLC) preparation when the skin is exposed to UV light, the effectiveness of which is comparable to that of Isopropyl palmitate and Vitamin C (Patimah *et al*., 2020).

Nanostructured Lipid Carrier (NLC) is a method to increase drug penetration through the stratum corneum because it has several advantages. One advantage is that solid lipids in the system can control drug release (Patimah *et al*., 2020). The NLC (Nanostructured Lipid Carrier) preparation must be tested for physical characteristics to ensure that the dosage formed has good specifications. One of the tests includes testing the zeta potential value (Patimah *et al*., 2020).

Zeta Potential testing is a method for assessing the stability of colloidal dispersions by reflecting the electrical charge on the surface of the particles. Particles with a zeta potential value more negative than -30 mV or more positive than +30 mV in the nanoparticle system indicate mutual repulsion (Patimah *et al*., 2020). Nanoparticles are particles with a varying size range, namely 50-1000 nm (Dewi *et al*., 2019).

NLC preparations containing the solid lipid myristic acid produce good drug absorption and release characteristics because myristic acid has an optimum carbon chain length. Myristic acid is a natural ester compound derived from nutmeg plant extract. Myristic acid is white, oily crystals with a faint odor. In research by Melania Agustin, Ayu (2022) entitled "The Effect of Myristic Acid

concentration on the Potential Zeta Value of Nanostructured Lipid Carriers (NLC) Coenzyme Q10" using concentrations of 7%, 8% and 9%, the results showed that if the solid lipid concentration was higher then it will affect the shape of the droplet size and the shape of the droplet size can influence the zeta potential value to be higher, which can cause the stability of a nanoemulsion preparation. So, in this study, myristic acid was used with concentrations of 10%, 11%, and 12%. Based on research conducted by Siti Aisiyah (2019), it is said that different types of solid lipids significantly influence particle size. Apart from that, it is based on research conducted by Ayu Wulan (2022), where a myristic acid concentration of 5% produces a dilute preparation, so this research is continuing by looking at the effect of variations in myristic acid concentration on the zeta potential and particle size of NLC Coenzyme Q10.

METHODS

Material & Tool

Coenzyme Q10 (Chemco), Span 80 (Sigma), Tween 80 (Sigma), Propylene glycol, Myristic Acid, Caprylic, Phosphate Buffer pH 6.0±0.2 prepared from sodium hydroxide and potassium dihydrogen phosphate (Merck) (Pro Analysis).

Analytical balance (ACIS AD-300i), pH meter, Weighing bottle, Measuring glass (Pyrex), Beaker glass (Pyrex), Hotplate magnetic stirrer (SCILOGEX MS-H280-Pro), Watch glass, Stirring rod, Porcelain cup, Test tube, Vortex, Drop Pipette, Particle Size Analyzer, Ultra Turax (IKA-T25).

FormulationNLC manufacturing

The Nanostructured Lipid Carrier (NLC) formula is made by melting solid (Myristic Acid) and liquid (Caprylic) lipids at a temperature (600 ± 50^{0}C) using a Hotplate. The lipid phase mixture was then stirred using a hotplate and magnetic stirrer at 1000 rpm for 1 minute at a temperature of (600 ± 50^{0}C). The active ingredient (Coenzyme Q10) was added and then stirred using a hotplate and magnetic stirrer at a speed of 1000 rpm for 2 minutes at a temperature of (600 ± 50^{0}C) until completely dissolved. Span 80, previously heated at a temperature of (600 ± 50^{0}C) was added and then stirred using a hotplate and magnetic stirrer at a speed of 1000 rpm for 1 minute at a temperature of (600 ± 50^{0}C). The water phase was made by mixing propylene glycol with phosphate buffer pH 6.0 ± 0.2, stirring using a hot plate and magnetic stirrer at 1000 rpm for 2 minutes at a temperature of (600 ± 50^{0}C). Tween 80, previously heated at a temperature of (600 ± 50^{0}C) was added and then stirred using a hotplate and magnetic stirrer at a speed of 1000 rpm for 1 minute at a temperature of (600 ± 50^{0}C). The lipid and oil phases were mixed and stirred using an ultra-tax high shear homogenizer at a speed of 6000 rpm for 3 minutes at a temperature of (600 ± 50^{0}C). The next stage is cooling. This stage is carried out by stirring the final mixture at 6000 rpm until the emulsion temperature reaches room temperature (Gusti Ebtavanny T, 2018).

Table 1. Coenzyme Q10 NLC formulation

Material	Concentration (%)*			Function
	F1	**F2**	**F3**	
Coenzyme Q10	1	1	1	Active substance
Tween 80	8	8	8	Surfactant
Span 80	12	12	12	Surfactant
Propylene glycol	10	10	10	Co-surfactant
Myristic Acid	10	11	12	Solid lipids
Caprylic	7	7	7	Liquid lipids
Phosphate buffer pH 6.0	ad 100	ad 100	ad 100	Preparation (water phase)

* Information:
F1: NLC Coenzyme Q10 preparation with 10% myristic acid concentration
F2: NLC Coenzyme Q10 preparation with a myristic acid concentration of 11%
F3: NLC Coenzyme Q10 preparation with 12% myristic acid concentrate

Characteristic Evaluation

Organoleptic

Testing was done by observing the shape, color, transparency, and phase formed from the nanoemulsion preparation. This observation is carried out visually using the five senses.

pH

pH testing was carried out using a Laqua Horiba Scientific pH meter, calibrated first using pH 4.00 and 7.00 buffers before being used to measure nanoemulsions and gel nanoemulsions.

Spread Power

The aim of measuring spreadability is to determine whether the sample can be spread evenly on the skin surface when applied. 1 g of the emulgel preparation was taken and then placed in the center between 2 round glass plates. The upper plate is loaded with a weight weighing 10 g for 1 minute. Observe the diameter of the sample distribution. This is done continuously until a constant diameter is obtained to see the effect of the load on changes in the diameter of the gel spread. The required spreadability for transdermal preparations is 5 cm-7cm (Tungadi, R., Pakaya, S.P., As'ali. P.D, (2023).

Particle Size and Zeta Potential Value

Particle size and Zeta potential Value testing were done using the Particle Size Analyzer Nanotrac Wave II (PSA). A 1-gram NLC sample was diluted using 10 ml of aqua bidestilata was then vortexed first before observing. The data observed are the average particle diameter and zeta potential.

Freeze ± Thaw Test

Storage in the freeze-thaw cycle was carried out to see the physical stability of the cream after being stored for thirty days at different temperatures, namely 40^0C and 40^0C. Storage is carried out in six cycles. The sample was weighed at 1 gram and kept at 40^0C (24 hours), then moved to 40^0C (24 hours) for one process simultaneously. Then, the organoleptic and phase separation, pH, dispersion, particle size, and zeta potential value were observed (Wardani *et al*., 2016).

Data Analysis Analysis

Data analysis used to determine the effect of solid lipid concentration on the physical characteristics of NLC Coenzyme Q10 used Data analyzed using the One-way ANOVA test with a sig value < 0.05, which means it is significantly different, and a sig value > 0,05.

RESULTS AND DISCUSSION

Table 2. Test results of physical characteristics of NLC Coenzyme Q10

Uji	Before *Frezee Thaw*			After *Frezee Thaw*		
	F1	**F2**	**F3**	**F1**	**F2**	**F3**
Organoleptic	Yellow cream, no phase separation	Yellow cream, no phase separation	Yellow cream, no phase separation	Yellow cream, no phase separation	Yellow cream, no phase separation	Krim berwarna kuning, tidak terdapat pemisahan fase
Spreadability (cm)	5,26	5,56	5,85	6,18	6,81	6,92
pH	5,85	5,71	5,57	5,80	5,68	5,50
Particle size (nm)	96,43	89,07	74.04	108,06	93,50	84,64
Zeta potential(mV)	(-) 35,49	(-) 33,34	(-) 30,74	(-) 33,20	(-) 31,54	(-) 28,69

Based on the data in Table 2, it is known that differences in solid lipid concentrations do not have much effect on the organoleptic properties of NLC coenzyme Q10 preparations. In testing the pH of preparations F1 to F3, variations in the concentration of solid lipids also do not affect the pH of the practices much. This is because, in the NLC formula, a buffer solution is added, which is used to buffer the pH of the preparations so that they remain at pH 6.0 ± 0.2. Even though the pH results of the practices varied, all formulas were still said to meet the desired specifications, namely pH 6.0 ± 0.2. The pH value range of topical preparations is adjusted to the pH stability of the active ingredients and the skin pH value (4.5-6.5) (Tranggono & Latifah, 2007). They were testing the spreadability of NLC Coenzyme Q10 with a load of 200g. They obtained results at F1 4.7cm, F2 5.4 cm, and F3 5.6 cm, so it can be concluded that F1 does not meet the range of spreadability specifications, namely 5 - 7 cm for topical preparations. F2 and F3 have met the specifications for good cream preparations (Tungadi, R., Pakaya, S.P., As'ali. P.D, 2023).

Determination of droplet size and zeta potential is the most commonly used method to assess the stability of preparations. Based on the results of size measurements, it is known that the increasing concentration of myristic acid can cause the particle size to become smaller. The particles of the NLC Coenzyme Q10 preparation before freeze-thaw, the results of which can be seen in Table 1, show that the smallest particle size obtained was 74.04 nm at F3, and the largest particle size was 96,43 nm on F1. Even though each formula has varying particle sizes, the resulting particle size still meets the specified specifications, namely nanoparticles in the value range of 20-200nm (Guan *et al*., 2016).

Test results for zeta potential values in F1-F3 nanoemulsion preparations with different concentrations (F1: 10%, F2: 11%, F3: 12%) resulting in a range of zeta potential values before freeze-thaw, namely F1 |-35,49mV|, F2 |-33,34mV| and F3 |-30,74mV| From the test results it can be seen that all formulas have a negative zeta potential value, this is due to the presence of free fatty acids in the Nanostructured Lipid Carrier (NLC) Coenzyme Q10 component (Shanmugam, et al., 2014), even though the three formulas have varying values but remain within the specification range, namely |± 20 mV| to |± 40 mV| (Murdock, R.C., 2008). When compared with research conducted by Ayu Melania (2022), with a myristic acid concentration of 7,8,9%, the resulting zeta potential value was |-22.38 mV|, |-26.78 mV| and |-29.61mV|. The result is that by increasing the concentration of myristic acid, the zeta potential value will decrease further. This is inversely proportional to my research, where increasing myristic acid concentration will reduce the zeta potential value. This is due to the increase in the ratio of solid lipids, which can increase viscosity and interfacial tension, which causes particle agglomeration effects followed by an increase in particle size (Prasetiowati *et al*., 2018). Suppose the zeta potential value in previous research is combined with the zeta potential value in this research. In that case, the data obtained will form a parabolic graph where adding a myristic acid concentration of 10% produces the highest zeta potential value. According to Murdock (2008), particles with zeta potential values greater than +30 mV or less than -30 mV usually have high stability (Murdock *et al*., 2008).

The stability test results of 6 cycles of NLC preparations showed that the homogeneity test of the three preparations remained homogeneous, did not experience phase changes, and no separation occurred. pH test on F1 (5.80), F2 (5.68), and F3 (5.50), based on the statistical test of pH F1, F2, and F3, there is no significant effect (significant p-value > 0.05) between the three formula before and after freeze-thaw treatment, the pH test results remained stable and entered the requirements of a suitable pH range, which means that the choice of buffer used was good enough to support the pH of the preparation while remaining within the specifications for topical preparations (Tranggono & Latifah, 2007).

The cream spreadability test on F1, F2, and F3 experienced an increase in spreadability. This could be caused during the storage time of the NLC preparation. The statistical test for the spreadability of F1, F2, and F3 showed a significant effect (significant p-value < 0.05) between before and after freeze-thaw treatment. However, the three NLC formulas still entered the good spreadability range (5 - 7 cm). In the statistical test of the F1 Potential Zeta Value, there is a significant influence (significant p-value < 0.05) between before and after freeze-thaw treatment; this is different from F2 and F3; the change in potential zeta value does not have a significant influence (significant p-value > 0.05). The particle size statistical test found that changes in particle size in F1 and F2 did not have a significant effect (significant p-value > 0.05). However, in F3, there was a significant effect (significant p-value < 0.05) between before and after treatment. Freeze thaw. The freeze-thaw process can be successful, and phase separation does not occur depending on the ability of the NLC to immediately recover from crystal water pressure. At a temperature of 40^0C, the water phase freezes and tends to shrink, narrowing the

water phase space and causing the oil globules to be close together. As a result, the viscosity of the preparation increases. In the thawing process or at a temperature of 40^0C, the crystals will melt and spread again. If the recovery speed of the NLC is slow, then instability can occur; this is what causes changes in the zeta potential value and the NLC particle size to become more prominent after passing the freeze-thaw test when compared to before the freeze-thaw process (Hamsinah *et al*., 2016).

CONCLUSION

Based on the research results, it can be concluded that the concentration of myristic acid affects spreadability with a significant p-value produced, namely 0.001 (significant p-value <0.05), but the concentration of myristic acid does not always affect particle size and zeta potential value. The concentration of myristic acid did not affect pH, with a significant p-value of 0.902 (significant p-value > 0.05).

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