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Effect of Alpha-Lipoic Acid on the Characteristics and Physical Stability of NLC-Green Tea Extract

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Abstract

Background*: The addition of alpha-lipoic acid in Nanostructured Lipid Carrier-Green Tea Extract (NLC-GTE) has potential to increase effectiveness of anti-aging preparations. It happened because alpha-lipoic acid can increase stability and antioxidant activity.* ***Objective****: Comparing the physical characteristics and stability of NLC-GTE with or without alpha-lipoic acid.* ***Methods****: NLC-GTE manufactured using the High Shear Homogenization method. NLC-GTE was divided into two formulas, without the addition of alpha-lipoic acid for F1 and with the addition of alpha-lipoic acid for F2. The characteristics and physical stability were tested, including organoleptic, pH, particle size, and polydispersity index. Stability test was held using the thermal cycling method.* ***Results****: Based on characteristic test, it was found that F2 had larger particle size value than F1. The average particle size value of F1 is 313.9 ± 0.76 nm and 423.4 ± 0.75 nm for F2. The F1 and F2 preparations had a polydispersity index value below 0.3, so they were homogeneous. The average pH value of F1 is 5.998 ± 0.01, and F2 is 4.798 ± 0.004. The physical stability test showed a difference before and after the sixth day in particle size and pH, but it was still in the range, so it was safe. However, there was a separation in F1 after day 6.* ***Conclusion****: Based on the characteristics and physical stability tests, F1 (without alpha-lipoic acid) and F2 (with alpha-lipoic acid) had differences in particle size and pH. From the physical stability test, it can be concluded that F2 is more stable than F1.*

Keywords*: nanostructured lipid carrier (NLC), green tea extract, alpha-lipoic acid*

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INTRODUCTION

At this time, the development of the world's population has entered a period called the aging population, an increase in life expectancy followed by an increase in the number of older people in all countries in the world. Indonesia experienced an increase in the number of older people from 18 million people (7.56%) in 2010 to 25.9 million people (9.7%) in 2019, and it is expected to increase in 2035 to 48.2 million people (15.77%) (Kemenkes, 2020). It will be the reason for health problems, including aging (Ahmad & Damayanti, 2018).

Aging is a decrease in the size and number of skin cells and changes in skin function resulting from a decline in the standard structure and function of normal skin. Physiological changes in elderly skin are shown in impaired barrier function, slowed epidermal cell turnover, decreased blood vessel network around the base of the hair and glands, decreased cell turnover function, and decreased sweat production (Anggowarsito, 2014). Cosmeceuticals are cosmetic products with active ingredients that aim to have medical or medicinal benefits and protect against deteriorating skin conditions. Cosmeceuticals claim to improve skin tone, function, and texture by promoting collagen growth and reducing wrinkles (Vaishali *et al.*, 2013).

Camellia sinensis is a plant that produces various types of tea, including green tea. Green tea extract has pharmacological effects, including antioxidants and photoaging, that have potential in developing cosmeceutical products (Ganesan & Dong, 2016). Green tea extract has a high solubility in water but very low solubility in lipids, making it difficult to penetrate the stratum corneum. Green tea extract contains the main catechins such as epicatechin gallate (ECG), epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG). The most active and abundant catechin in green tea is epigallocatechin gallate (EGCG) (Namita & Kumar, 2012).

EGCG is highly soluble in water (40 g/l at 20^0C) and has good stability at pH 3.7 (25^0C) and pH 3.9 (40^0C). EGCG has low lipophilicity, with a log P value of 1.1 at pH 4.0, while the optimal log P value for optimal penetration ranges from 2 - 3 (Rosita *et al.*, 2019). A nanocarrier system can be used to overcome this problem. Nanocarrier consists of Nanoparticle (NP), Solid Lipid Nanoparticle (SLN), and Nanoparticle Lipid Carrier (NLC). When compared in cost, SLN and NLC are more affordable than Liposomes. In addition to being more expensive, liposomes have the disadvantage of being quite complex in preparation and low instability (Manea *et al.*, 2014). Meanwhile, SLN and NLC showed high drug loading capacity (Frias *et al.*, 2016). SLN only consists of solid lipids that form perfect crystallinity. Thus, the entrapment power (EE) is lower. Meanwhile, NLC is a modification of SLN, which forms lower crystallinity so that EE is higher and has better stability than SLN (Frias *et al.*, 2016). It is because NLC contains liquid lipids, which can reduce crystallinity to accommodate more active ingredients (Mayangsari *et al.*, 2021).

Green tea extract also has a disadvantage, known as photodegradation which causes it is easy to lose active compounds, reducing stability. In the research of Chen *et al.* (2017), EGCG has a deficiency, which is proven with the presence of reactivity that causes oxidation, hydrolysis, epimerization, and polymerization reactions. To overcome this, needed the addition of co-antioxidants (Scalia *et al.*, 2013). One of the co-antioxidants that can be used is alpha-lipoic acid, where alpha-lipoic acid has antioxidant activity that effectively removes free radicals by reducing alpha-lipoic acid to dihydrolipoic acid. Alpha-lipoic acid is soluble in fat and water to reach all parts of the cell (Perricone, 2000).

Alpha-lipoic acid is a weak acid with a pKa of 4.7, therefore it will affect the characteristics of the preparation (Cichewicz *et al.*, 2013). In addition, alpha-lipoic acid is an antioxidant that can keep the preparation stable. Alpha-lipoic acid has the ability neutralize free radicals in watery and fatty areas of cells. Alpha-lipoic acid can neutralize various free radicals, such as singlet oxygen, superoxide, peroxyl and hydroxyl radicals. In the study of Hu *et al.* (2015), alpha-lipoic acid can reduce lipid oxidation in order to protecting EGCG from degradation during carrier process. EGCG can be degraded by pH so acidic state is needed to be stable. Alpha-lipoic acid is a weak acid with a pKa of 4.7, it will lower the pH and increase the stability of EGCG.

In a study by Bianchi *et al.* (2013), alpha-lipoic acid as a photo stabilizer for EGCG was much more efficient and effective at lower concentrations than vitamins E, C, and BHT (*Butylated hydroxytoluene*). Alpha-lipoic acid inhibits EGCG photodegradation and maintains functional activity under solar radiation. The addition of alpha-lipoic acid in NLC green tea extract can increase the effectiveness of anti-aging preparations. It happened because alpha-lipoic acid can increase stability and increase antioxidant activity. For the introduction in this preparation, the characteristics and physical stability tests were carried out on NLC-Green Tea Extract and NLC-Green Tea Extract with 0.5% alpha-lipoic acid.

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The preparation of this research using high shear homogenizer (HSH) method, in order to avoid the use of organic solvents that needed in the other technique.

MATERIALS AND METHODS

Materials

The ingredients used in this study were Meditea Green Tea Extract (PT. Angler BioChemab, Indonesia), cetyl palmitate (BASF, Indonesia), glyceryl stearate (BASF, Indonesia), poloxamer 188 (PT. Megasetia Agung Kimia, Indonesia), lecithin (Solae, England), grape seed oil (Brighton, UK), tween 20 (Zhang Yan, Singapore), alpha-lipoic acid (Tokyo Chemical Industry, Tokyo, Japan), NaH_2PO_4 (SAP, Indonesia), and Na_2HPO_4 (SAP, Indonesia).

Instrumentation

This research used Ultra Turrax IKA®T25 Digital High Shear Homogenizer, OHAUS analytical balance, Beckman Coulter Delsa^{TM} Nano C Particle Analyzer, Thermo Scientific Hotplate, SI Analytics Lab 865 pH meter, and other supporting tools.

Methods

NLC-GTE manufacture

The manufacture of NLC-GTE used the HSH (High Shear Homogenizer) method consist of an oil phase and a water phase mixed. The oil phase consisted of solid lipids (cetyl palmitate and glyceryl stearate) and liquid lipids (grape seed oil), melted at 70^0C for 30 minutes. In the 20th minute, the green tea extract, which was previously dissolved in a pH 5.0 buffer phosphate, was heated at the same temperature. After 30 minutes, the green tea extract liquid was added gradually into the oil phase and then stirred using a hotplate stirrer at 300 rpm for 10 minutes. The aqueous phase consisted of surfactants (tween 20), co-surfactants (Lecithin and Poloxamer 188), and buffer pH 5.0. All ingredients were put into a beaker and heated at 70^0C for 22 minutes. After that, the aqueous phase was homogenized using Ultra Turrax IKA®T25 Digital High Shear Homogenizer at 15000 rpm for 2 minutes. After finished with homogenization, the water phase was added to the oil phase and stirred using Ultra Turrax IKA® T25 Digital High Shear Homogenizer at 16000 rpm for 7 minutes. It was continued with stirring using a hotplate stirrer at 300 rpm for 12 minutes or until reaching room temperature. In F2 preparations, alpha-lipoic acid is added to the oil phase gradually while stirring using a hotplate stirrer. The formula can be seen in Table 1.

Physical characteristic testing

Organoleptic observations

Organoleptic observations were carried out by conducting color, odor, consistency, and visual phase separation tests.

Particle size and polydispersity index (PI)

Particle size and polydispersity index (PI) were measured using the Delsa^{TM} Nano C Particle Size Analyzer. The F1 and F2 preparations were diluted first by weighing 50 mg of the preparation plus 50 mL of distilled water and then stirred with a 500 rpm hotplate stirrer for 10 minutes. After that, the preparation was diluted again by taking 2 mL of the preparation and adding 8 mL of distilled water. The solution was stirred again at 100 rpm for 10 minutes. Lastly, the solution was put into a cuvette and measured using a Delsa^{TM} Nano C Particle Size Analyzer.

pH value measurement

pH testing was performed by measuring the preparation using a pH meter which was previously calibrated with a standard solution of pH 4. The name of the tool used is SI Analytics pH Meter Lab 865.

Tabel 1. F1 and F2 test formula

Composition	Material Function	Amount used (%)	
		F1	**F2**
Green tea extract	Active Ingridients	0.1	0.1
Cetyl Palmitate	Solid Lipid		
Glyceryl Stearate		1.16 : 1.16 : 1 (10%)	
Grape Seed Oil	Liquid Lipid		
Tween 20	Surfactant	2	2
Lecithin	Co-surfactant	1:1	
Poloxamer 188		(1%)	
Alpha-lipoic acid	Co- antioxidant	-	**0.5**
Water	Solvent	Ad 100	Ad 100

*F1 = NLC-GTE without Alpha-lipoic acid
F2 = NLC-GTE with Alpha-lipoic acid

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Physical stability test

Physical stability testing was carried out using the thermal cycling method. The preparation was stored in 1,5 cycles, two days in the oven at 40^0C , in the refrigerator at 2 - 8^0C, and in the oven for six days. Organoleptic, pH, particle size, polydispersity index, and the presence or absence of separation testing was carried out, before and after the physical stability testing.

RESULTS AND DISCUSSION

Physical characteristic test

Organoleptic observation

In the manufacturing process of NLC-GTE preparations with two formulas, NLC-GTE without the addition of alpha-lipoic acid for F1 and with the addition of alpha-lipoic acid for F2. The results of the characteristic test showed that the NLC-GTE preparations (F1 and F2) had white color for F1 and broken white for F2, odorless, liquid consistency, and soft texture. Visually, the homogeneous NLC-GTE preparations can be seen in Table 2.

Particle size and polydispersity index were important factors in the parameter characteristics of nanoparticle preparations. The average particle size of NLC GTE was 313.9 ± 0.76 nm for F1 and 423.4 ± 0.75 nm for F2. A statistical test was performed using the Paired-Sample T-Test with $\alpha = 0.05$, and the result was the Sig value. (2 tailed) < 0.05, it can be concluded that there was a significant difference. The particle size of F2 was larger than F1 due to the addition of alpha-lipoic acid. It was supported by Lason *et al.* (2017) research that the particle size of the NLC base without alpha-lipoic acid was smaller than the NLC preparation containing the active ingredient alpha-lipoic acid. However, the particle size of F2 was still within the range for NLC preparations, below 1000 (Mayangsari *et al.*, 2021). For the results of the polydispersity index, the results were below 0.3, i.e., 0.263 ± 0.007 for F1 and 0.272 ± 0.033 for F2. With the addition of alpha-lipoic acid, the result showed that the greater the concentration, the greater the value of polydispersity index, but it is still below the range therefore the formula is still homogeneous. The literature of Lason *et al.* (2017) stated that a polydispersity index value below 0.3 means it was homogeneous. For statistical test using Paired-Sample T-Test with $\alpha = 0.05$, the value of Sig. (2 tailed) > 0.05, it can be concluded that there was no significant difference between F1 and F2.

The average pH value of F1 was 5.998 ± 0.01, and F2 was 4.798 ± 0.004. It was in line with the literature, i.e. 4.5 - 6.8 (Mayangsari *et al*., 2021). The NLC-GTE preparation with the addition of alpha-lipoic acid has more acidic pH. After statistical testing with the Paired-Sample T-Test with $\alpha = 0.05$, information was obtained that the value of Sig. (2 tailed) < 0.05, it can be concluded that there was a significant difference. Differences in the concentration of alpha-lipoic acid between formulas affect the pH value, which make the preparation relatively stable under acidic conditions. This can protect the preparation from being degraded by pH, resulted in the preparation remains stable. The addition of alpha-lipoic acid which is a weak acid with a pKa value of 4.7 resulted in a conclusion that the greater the concentration, the lower the pH value (Cichewicz *et al.*, 2013). However, all formulas still meet the normal pH range of the skin (4.5 – 6.8) so it does not cause any side effect on the skin.

Physical stability testing

Physical stability testing used the thermal cycling method by storing the preparations in 1.5 cycles, two days in the oven, in the refrigerator, and in the oven for six days. Organoleptic, pH, particle size, polydispersity index, and the presence or absence of separation testing was carried out, before and after the physical stability testing.

Tabel 2. Characteristic observation results of F1 and F2

Observation		F1	F2
Organoleptic	Color	White	White
	Consistency	Liquid	Liquid
	Texture	Soft	Soft
	Odor	Odorless	Odorless
	Particle Size	313.9 ± 0.76	423.4 ± 0.75
	Polydispersity index	0.263 ± 0.007	0.272 ± 0.033
	pH	5.998 ± 0.01	4.798 ± 0.004

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Table 3. Results of observation of particle size, polydispersity index, pH, and the presence or absence of separation in the physical stability test

Formula	T = 0				T = 6 days			
	Separation	Particle Size (nm) ± SD	PDI ± SD	pH ± SD	Separation	Particle Size (nm) ± SD	PDI ± SD	pH ± SD
F1 (Without Lipoic)	-	313.9 ± 0.76	0.263 ± 0.007	5.998 ± 0.01	+	385.26 ± 2.49	0.265 ± 0.012	5.730 ± 0.007
F2 (With Lipoic)	-	423.4 ± 0.75	0.272 ± 0.033	4.798 ± 0.004	-	435.16 ± 3.65	0.260 ± 0.024	4.847 ± 0.012

Information:
- = absence of separation
+ = presence of separation

After six days, there were no significant changes in color and odor based on organoleptic testing. However, in F1, there was separation after six days, while in F2, there was no separation, so it remained homogeneous. It meant that F1 was unstable at extreme temperatures while F2 remained stable. In the physical stability test, F2 contains alpha-lipoic acid and resulted with no phase separation occurs. This is supported by the research of Lason *et al*. (2017), that NLC containing the active ingredient alpha-lipoic acid when observed with an optical microscope showed that the preparation was homogeneous and no agglomerates or crystals were found.

Observations of particle size values in F1 and F2 were conducted on days 0 and 6, resulted that there was a change in particle size but still according to the literature, which has a value below 1000 nm (Mayangsari *et al*., 2021). In F1 and F2, statistical tests were carried out using the Paired-Sample T-Test with α = 0.05. The results obtained were the Sig values (2 tailed) < 0.05, it can be concluded that there was a significant difference before and after 1.5 cycles of thermal cycling. This difference is indicated by an increase in particle size before and after storage.

The results of the observation from the F1 and F2 polydispersity indices after the sixth day were below 0.3, and this was in accordance with the literature (Lason *et al*., 2017), if the value was below 0.3, the preparation was homogeneous. The polydispersity index before and after 1.5 cycles of thermal cycling in F1 and F2 was statistically tested using the Paired-Sample T-Test, and the results obtained were Sig values. (2 tailed) > 0.05, so there was no difference and the preparation remained stable.

Related to pH observation on days 0 and 6, the results were relatively stable for F1 and F2. The pH value obtained is still in the skin pH range of 4.5 - 6.8 (Mayangsari *et al*., 2021). F1 and F2 were statistically tested with the Paired-Sample T-Test, and the results obtained were Sig values (2 tailed) < 0.05, so there was a significant difference in pH F1 and F2. The results of observations for particle size, polydispersity index, pH, and the presence or absence of separation were in Table 3. However, all formulas were still in the skin range of 4.5 - 6.8 so it still safe to be used. ALA is a weak acid with a pKa of 4.7 therefore it can lower the pH of the formulation but increase the stability of EGCG.

CONCLUSION

Based on the physical characteristics test, it was concluded that F1 (without alpha-lipoic acid) and F2 (with alpha-lipoic acid) had differences in particle size and pH. From the physical stability test, it can be concluded that F2 is more stable than F1.

AUTHOR CONTRIBUTIONS

Conceptualization, F. Y. A., W. S., D. A. P.; Methodology, F. Y. A., W. S., D. A. P.; Validation, F. Y. A., W. S., D. A. P.; Formal Analysis, F. Y. A.; Investigation, F. Y. A.; Resources, F. Y. A., W. S., D. A. P.; Data Curation, F. Y. A., W. S.; Writing - Original Draft, F. Y. A.; Writing - Review & Editing, F. Y. A., W. S., D. A. P.; Visualization, F. Y. A.; Supervision, F. Y. A., W. S., D. A. P.; Project Administration, F. Y. A., W. S., D. A. P.; Funding Acquisition, F. Y. A.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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