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Development Nanostructured Lipid Carriers (Nlc) Loaded Resveratrol with Different Combination of Soybean Oil and Oleic Acid

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ABSTRACT

Resveratrol is a potential antioxidant to improve skin aging. However, resveratrol has the disadvantage of being insoluble in water and unstable to light, for this reason is made the delivery system of lipid nanostructure carrier that has the ability to protect unstable compounds that can increase the stability of active ingredients during storage period. The purpose of this study was to formulate resveratrol nanostructure lipid carrier (NLC) and evaluate the stability of nanoparticles in maintaining stability during storage. The NLC formula is made with different comparative concentrations of liquid lipids, soy bean oil and oleic acid (3: 1, 2: 2, 1: 3) using the High Shear Homogenization method. Characterization is then carried out. NLC includes particle size, potential zeta, polydispersity index, viscosity, PH, entrapment efficiency of resveratrol, FTIR, NLC morphology and storage stability test for 60 days with particle size parameters, polydispersity index and PH. NLC resveratrol with a ratio of 1: 3 shows spherical morphology with an average particle size of 304.36 ± 35.82 nm, polydispersity index 0.243 ± 0.03 , zeta potential -47.9 ± 1.26 and entrapment efficiency 95.08 ± 0.09 , viscosity 420.00 ± 16.3 . In the stability test of NLC-F3 with a ratio of 1: 3 is stable for 60 days of storage including particle size, polydispersity index and PH.

Key Words: Resveratrol, NLC, Cream, Anti-aging, Stability

1. INTRODUCTION

Photo aging is skin aging caused by exposure to excessive UV radiation on the skin, aging of the skin will result in a decrease in skin quality which is characterized by the appearance of wrinkles, pigmentation, actinic keratosis, hypopigmentation, decreased skin elasticity and rough skin texture (Christian et al, 2004 ; Puizina, 2008). To slow down the skin aging process, prevention and repair efforts need to be done. These efforts can be done through cosmetics that can come from hormones, topical growth factors, and antioxidants that are expected to induce collagen synthesis, thereby improving wrinkles and skin texture (Ekawati, 2015). One of the potential antioxidants to improve skin aging is resveratrol (Baxter, 2008; Ndyiae et al, 2011). Not only as an antioxidant, resveratrol also has anti-inflammatory, antiproliferative and skin-lightening activity (Gocke et al, 2012; Farris, 2014). Resveratrol as an antioxidant has several disadvantages, namely the low solubility of water and an ingredient that is unstable against light (Route et al, 2013; Zupancic et al, 2015). For this reason, a topical delivery system is needed that can increase solubility, protecting the stability of active ingredients during storage. Topical delivery system with a lipid base carrier can improve the stability of active and active ingredients, including solid lipid nanoparticles (SLN), SLN is a new generation of solid lipid emulsions that have relatively small particle size, large surface area, can protect unstable compounds, can control the release of active substances, biodegradable and non-toxic (Rosita et al, 2013; Amalia et al, 2015). SLN has several disadvantages such as low entrapment capacity and drug discharge in the system during storage (Pezeshki, 2014). To overcome the weaknesses of the SLN, the second generation of nanoparticle lipid carrier (NLC) nanoparticles was developed. Besides being able to overcome the weaknesses of SLN, NLC can also increase penetration, reduce irritation and can increase the stability of active ingredients during

storage (Lhi and Zhi, 2011). This study aims to create and characterize NLC resveratrol and conduct stability and irritability studies.

2. MATERIALS AND METHODS

Materials used in this study if not stated otherwise have pharmaceutical grade purity. The main ingredient used in this study is resveratrol derived from Naturalin Bio Resources (China), cetyl palmitate (BASF, Germany), soybean oil (Natures, India) and oleic acid (PT. Brataco, Indonesia), Propilenglycol (Dow Chemical Pacific, Singapore), Polysorbate-80 (Kao Corporation, Tokyo), Ethanol pa (Merck, Germany), buffer Acetat pH 5.0 ± 0.05.

AIAT: Particle size analyzer (Delsa Nano™ particle size analyzer), spectrophotometer, centrifuges, refrigerators, electron transmission microscopes, magnetic stirrers.

2.1 Methods and Manufacturing

Resveratrol was dissolved in soybean oil and oleic acid, the mixture was added in lipid setyl palmitate, then stirring with a magnetic stirrer 5000 rpm 3 minutes at 60°C, then added to the mixture tween 80 and hot propylene glycol was stirred with magnetic stirrer 5000 rpm for 3 minutes, the next step was added acetate buffer pH 5 ad 100 ml stirring with a magnetic stirrer 5000 rpm until an emulsion was formed, an o / w emulsion mixture was homogenized at a speed of 16,000 rpm (Ultra Turrax T-25 IKA Laborotechnik) for 3 minutes 5 cycles . The last stage was emulsified at a speed of 500 rpm at room temperature until cold and formed an NLC system. Characterization

Table I. Composition of NLC resvetrol formula

Composition (%b/b)	Formulation		
	NLC-F1 (%)	NLC-F2 (%)	NLC-F3(%)
Resveratrol	1	1	1
Setil palmiat	15	15	15
Sobean oil	11.25	7.5	3.75
Oleic acid	3.75	7.5	11.25
Tween 80	20	20	20
Propilenglycol	20	20	20
Dapar acetat ph 5	100	200	100

Noted: Formula NLC-F1 comparison of soybean oil: 1: 3 oleic acid, Formula NLC-F2

Comparison of soybean oil: oleic acid (2: 2). Formula of NLC F-3 soybean oil: oleic acid (3: 1)

2.2 Physical Evaluation of Cream

Preparations Organoleptic observations of NLC include odor, color, homogeneity

2.3 Characterization of NLC

Resveratrol Characterization tests on NLC resveratrol include particle size analysis, zeta potential and entrapment efficiency measurements.

2.4 Determination of Particle Size and Polidispersity Index (PDI)

Inspection of particle size and polydispersity index (PDI) diameter of NLC resveratrol system using Delsa™ Nano Sub Micron Particle Size Analyzer. NLC is dispersed with distilled water (1:40) then put into the cuvette. Next, the sample filled cuvette is inserted into the sample holder. The particle size analyzer will measure the sample for 10 minutes. The Polydispersity Index (PI) describes variations in a sample. PI values less than 0.3 indicate that the monodispersion sample. (Das and Anumita, 2011).

2.5 Determination of Viscosity

Measurements using a Rion viscosity meter. Using rotor no. 2. 100-4000 dps (using 400-500ml glass beker sample container). A 150 gr Nanostructure Lipid Carrier (NLC) is inserted in the container, attach the rotor to the container, make sure it is near the bubble and spread evenly on the rotor surface. Then the viscosity is ignited and left for a while until the reading is stable.

2.6 Zeta potential measurement

Zeta potential value was measured using a zeta potential analyzer at 25 ° C. The sample is diluted using distilled water before analysis. A colloid can be expressed as stable when storing if the zeta indigo potential is greater than +30 mV or less than -30 mV (Loo et al, 2012)

2.7 Trapping Efficiency

Measuring entrapment efficiency using a membrane filter with a molecular weight of 12000 kDa cut-off -14000 kDa. Lipid nanoparticles were inserted in the membrane filter and centrifuged 500 rpm for 7 hours. Then the supernatant obtained was filtered using whatman membrane filter with a diameter of 0.2 µm, then the number of active ingredients trapped in the NLC was calculated by the formula:

$$EE (\%) = \frac{W_i - W_f}{W_i} \times 100\%$$

2.8 FTIR (Fourier Transform Infra Red)

Spectrophotometer Infrared examination using Fourier transform infrared spectrometer (Jasco 4200, Germany). Resveratrol, soybean oil, oleic acid, sample NLC, are dispersed in (1: 1) dry Kbr powder then compressed or compressed to a hydraulic press equipped with a steam vaporizer to obtain a translucent plate. The plate is scanned at a wavelength of 400-4000 cm.

Transmission electron microscopy (TEM)

Droplet morphology is seen using Transmission Electron Microscopy (TEM). Three drops of the sample are placed in a copper lattice coated with carbon which has been dried at room temperature, carried out at a voltage of 120 KVA. (Gocke et al, 2012)

3. RESULTS AND DISCUSSION

Making NLC resveratrol emulsions, using the High Shear Homogenization method. This method was chosen because it is in accordance with lipophilic drugs, the principle of this method is to mix the fused lipid phase containing the active substance with surfactant which is heated at a temperature of 5-10 °C above the melting point of the lipid, using high speed mechanical stirring so that friction occurs. between particles which will cause a reduction or reduction in particle size (Amalia et al 2015, Svani et al, 2012). This method is the youngest method, because it does not require organic preservatives (Naseri et al, 2015).

3.1 Characterization of NLC resveratrol.

3.2 Determination of Particle Size and Particle Distribution

Particle size with Delsa Nano Particle Size Analyzer. From the examination of particle size (table: 2). On particle size examination of NLC-F1 containing a combination of liquid lipids, soybean oil and oleic acid with a ratio of soybean oil concentration, it produced a larger particle size of NLC-F1 863.50 ± 74.75 compared to NLC-F2 $518.00 \pm 45, 72$ and NLC-F3 304.36 ± 35.82 , in this case the energy in the homogenization process is the same in all NLCs, but the dispersed phase increases by 20-30%, causing a lack of available energy per lipid unit, which results in more particle size big. This is consistent with the study of Teeranachaideekul et al and Phipps, that high lipid concentration is directly proportional to the size of the particle size and when associated with the results of storage stability data, the particle size of F1 formula is unstable which is indicated by an increase in particle size during storage. The next step is to examine the particle size distribution. In particle size distribution results, there were no significant differences in polydispersity index (PI) values of all NLC formulas so that it can be concluded that the particle size distribution is homogeneous, in this case in accordance with the requirements of less 0.3 PDI value declared homogeneous (Das and Anumita, 2011) .

3.2 Zeta Potential Determination

In the potential zeta determination all formulas show a value between 47.6 - 57.2, if all particles in the suspension have a more positive PZ +30 mV or more negative -30 mV then it is stable. In the NLC formula F2 shows the highest zeta potential value among all formulas. Formula F2 is the same amount of liquid lipid as soybean oil and oleic acid in the same amount, where both liquid lipids are unsaturated fatty acids have a similar carbon chain double bond, and if combined will produce a high degree of repulsion between the same charged particles, and prevent flocculation. This is in accordance with the DLVO (Derjaguin – Landau – Verwey – Overbeek theory) theory that a system can be said to be stable if electrostatic repulsion dominates the van der wall force.

Table 2 : Result data organoleptic examination of NLC resveratrol

Sample NLC Resveratrol (soybean oil: oleic acid)	Organoleptic examination			
	Colour	Flavor	Consistent	Textur
NLC-F1(3:1)	White	No flavor	Semisolid	Soft
NLC-F2(2:2)	White	No flavor	Semisolid	Soft
NLC-F3(1:3)	White	No flavor	Semisolid	Soft

Table 3: Result analysis of size particles, indeks polidespersi,PH and viscositas resveratrol

Sample NLC resveratrol (Soybean oil: oleic acid)	Size particles (nm)	PDI	PH	Viscositas
NLC-F1(3:1)	863,50 ± 74.75	0.385 ± 0.05	5.01 ± 0.04	380.00 ± 8.16
NLC-F2(2:2)	528.00 ± 45.72	0.241 ± 0.02	4.85 ± 0.03	463.30 ± 12.4
NLC-F3(1:3)	304.36 ± 35.82	0.243 ± 0.03	4.84 ± 0.03	420.00 ± 16.3

Efficiency Determination The entrapment of resveratrol in an NLC system using a spectrophotometer On examination of entrapment efficiency, in Figure 2 the results of all formulas exceeded 90%, the high efficiency of entrapment is probably due to the high solubility of resveratrol against lipids, the liquid lipid content of soybean oil and oleic acid can trap the drug in high amounts and can reduce drug crystallization. (Lhi and zhi, 2012).

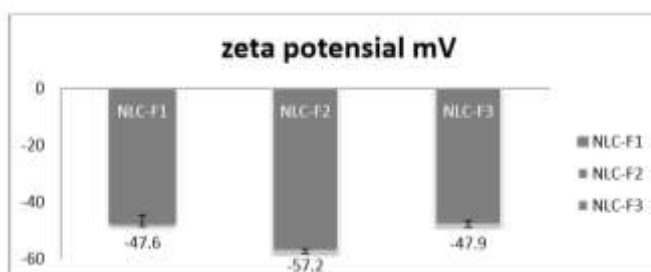


Figure 1: The average zeta histogram of the formula F1, F2 and F3 potential of the NLC resveratrol system is an average of three replications ± SD

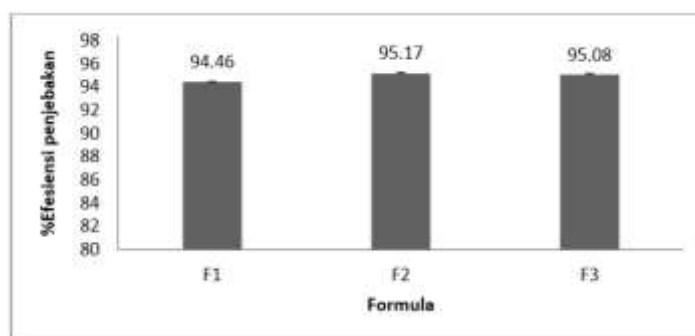


Figure 2: The efficiency of trapping F1, F2 and F3 formulas NLC resveratrol is an average of three replications.

2.3 Fourier transform infrared spectroscopy (FTIR)

Examination results In this study using FTIR spectroscopy to determine the possibility of an interaction between the active ingredient and additional material at the level of the functional group. Based on FTIR examination results in Figure 4. The

resveratrol NLC formula has an FTIR spectrum profile that is identical to resveratrol, this indicates that resveratrol is dispersed in the lipid matrix and there is no chemical interaction between resveratrol and lipid matrix.

4. RESULTS OF TRANSMISSION ELECTRON MICROSCOPY (TEM)

On microscopic examination using Transmission Electron Microscopy (TEM) showed spherical resveratrol NLC particles with a particle size of 50200 nm and a smooth particle surface. The spherical shape has greater solubility in the water phase, so it can increase the bioavailability of low solubility ingredients. (Chatterjee et al, 2017). The results of TEM analysis can be seen in Figure 4.

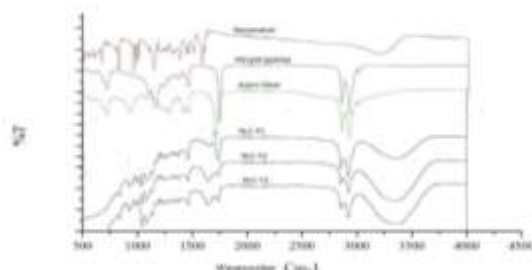


Figure 3 Results of FTIR spectroscopy examination of NLC RSV F1 (1: 3), NLC -F2 (2: 2), NLC-F3 (1: 3)

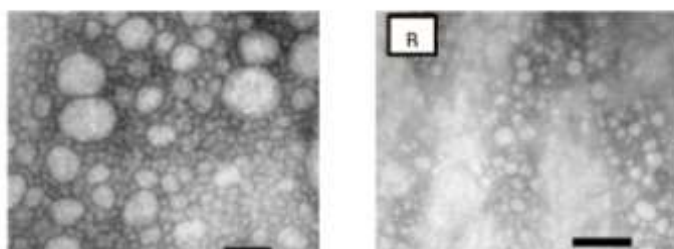


Figure 4. TEM NLC micrograph resveratrol formula F2 (A) scale of 50 nm and magnification of 10,000 x, (B) scale of 200 nm and magnification of 20,000 x.

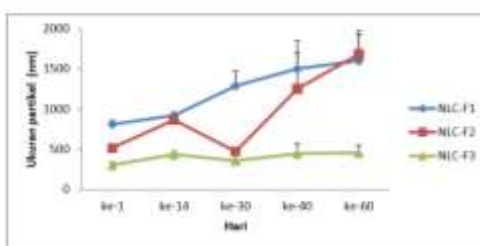


Figure 5 Results of particle size measurements of RSV F1, F2, F3 on day 1, 14th, 30th, 50th, 60th.

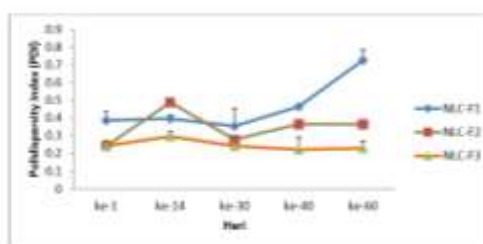


Figure 6 Measurement results of the NLC RSV F1, F2, F3 polidispersity index on the 1st, 14th, 30th, 50th, 60th days.

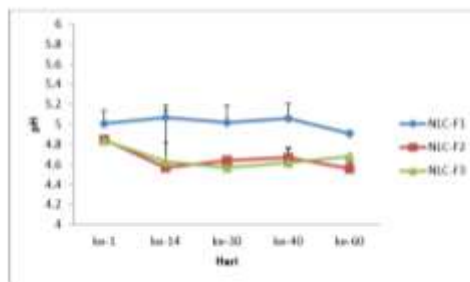


Figure 7 Histogram of RSV F1, F2, F3 pH measurement results on day 1, 14, 30, 50, 60th.

The next step was to examine the physical stability of the resveratrol NLC system for 60 days. In this test the particle size, particle size distribution and pH, particle size and particle size distribution were selected as stability parameters for nanoparticles because they play an important role in evaluating the colloidal shape during storage. From the results of examination of particle size stability in Figure 5, the results of the F3 formula were more stable than the formula F1 and F2. Size instability in formula F1 is associated with data viscosity. Viscosity of formula F1 is lower than formula F2, F3 low viscosity affects the decrease in stability (Raymundo et al, (2001). In addition, according to the law of sedimentation theory Stokes states that viscosity values are inversely proportional to sedimentation speed (Sinko, 2011). the speed of the formation of agglomerates so that the system with a lower viscosity will be easier to agglomerate. In the formula F4 is unstable during the storage period, when viewed from the storage data there is an increase in particles over time, this is related to the oswald ripening theory of particle growth, where large particles grow to sacrifice small particles causing a shift in particle size and particle size distribution to be greater. In the polydispersity index (PDI) stability test of figure 6, NLC-F1 is not stable compared to F2 and F3. NLC-F1 particle which is unstable so that it affects the instability of the particle size distribution (PI). Furthermore, for the pH stability test of NLC resveratrol, pH was obtained that all formulas were stable and filled the pH range of skin 4-6 (Ali and Yosipovitch, 2013).

3. CONCLUSION

Resveratrol NLC formula which shows the best characteristics is the NLC RSV formula with a ratio of 1: 3 where oleic acid concentration is greater than soybean oil with particle size 304.36 ± 35.82 nm, polydispersity index 0.243 ± 0.03 and entrapment efficiency $95,08 \pm 0.09$, viscosity 420.00 ± 16.3 . Zeta potential was -47.9 ± 1.26 , then physical stability examination was carried out for 60 days, obtained the results of the NLC RSV formula with a ratio of 1: 3 stable during 60 days storage including particle size 457.00 ± 94.31 , polydispersity index 0.230 ± 0.04 and pH 4.68 ± 0.02 . Thus the NLC comparison ratio (1: 3) has good character and stability for anti aging cream preparations.

SUGGESTIONS

It is necessary to test the resveratrol penetration in vivo so that it can be ascertained that NLC resveratrol is able to penetrate through the stratum corneum and target active substances in the skin.

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