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Characterization and Penetration Profile of Resveratrol-loaded Nanostructured Lipid Carrier (NLC) for Topical Anti-aging

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Keywords: Resveratrol, Nanostructured Lipid Carrier (NLC), Characterization, Penetration Profile, Anti-aging

Abstract. Skin aging is an essential factor in the degradation of skin's structural components, which is signed by less elastic and drier, lines and wrinkles, and looks and feels like older skin. Resveratrol (RSV) is a polyphenol compound with a potent antioxidant and antiaging effect, which can be found in many plants, such as red wine, red grapes, peanuts, berries, and Japanese knotweed root. It is active in inhibiting and neutralizing the formation of ROS and synthetic radicals under in vitro conditions. RSV is an unstable to light compound and insoluble in water, which has to be protected by proper delivery systems such as Nanostructured Lipid Carrier (NLC). This study aimed to determine the characterization and penetration profile of RSV-loaded NLC (RSV-NLC) using the High Shear Homogenization (HSH) method. RSV-NLC was formulated with four different surfactant ratios (5:5; 6:6; 7:7; 8:8) and characterized for entrapment efficiency, organoleptic, viscosity, pH, zeta potential, melting temperature, crystal lattice analysis, particle morphology, particle size, and Polydispersity Index (PI), then examined for their penetration profile. The different ratios of Tween 80 and Span 20 affect the characteristics of RSV-NLC (viscosity, zeta potential, particle morphology, and entrapment efficiency) but do not affect the penetration depth of RSV in the skin. Tween 80:Span 20 (5:5) is the best combination for RSV-NLC in characterization and penetration profile.

Introduction

Human skin is an active organ with various physiological function which contributes to personal beauty and attractiveness to others. External factors, like excessive ultraviolet radiation (UV) from sun exposure and pollution, contribute to signs of skin aging or as known as photoaging, which is accompanied by various visible morphological changes and invisible physiological dysfunction [1,2]. They lead to an increased formation of free radicals and represent up to 90% of the visible aging of the face's skin, which is signed by less elastic and drier, lines and wrinkles, and looks and feels like older skin [3,4]. All these photoaging processes are initiated by reactive oxygen species (ROS). ROS are generated constantly in the skin, causing damage to the collagen connective tissues and reducing fibroblasts' size [3,5]. One treatment used is antioxidants to cope with oxidative stress and cell damage due to UV exposure [1].

Resveratrol (RSV) (3,4',5-trihydroxy-trans-stilbene) is a polyphenol phytoalexin compound found in various plants, including red wine, red grapes, peanuts, berries, and Japanese knotweed root. In cosmetic products, RSV is used as an active ingredient having anti-inflammatory and antioxidant activities. RSV is active in inhibiting the formation of Reactive Oxygen Species (ROS), and under in vitro conditions, and neutralizing the synthetic DPPH and AAPH radicals [6]. RSV can scavenge superoxide anion radical (O₂⁻), hydroxyl radical (•OH), nitric oxide (NO•), and peroxynitrite (ONOO⁻) [2]. The in vitro studies using the Oxygen Radical Absorbance Capacity (ORAC) test showed that RSV has 17 times higher antioxidant activity (4845 µm vitamin E/g) compared to the synthetic coenzyme Q10-idebenone (279 µm moles of vitamin E/g). In contrast, the antiradical activity of RSV relative to peroxide radicals was higher than catechins, gallic acid, and ellagic acid [6]. Several other signs of aging have been described to be improved by RSV by increasing epidermal

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Introduction

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7 hydration, skin elasticity, skin thickness, reducing skin wrinkles, augmenting the content and quality of collagen, and the level of vascularization [3].

RSV can be found as both *cis* and *trans* isomeric forms, being *trans*-isomer the most abundant and biologically active form. *Trans*-RSV is a highly photosensitive compound being rapidly converted to the *cis*-form when exposed to light [7]. RSV properties must be considered since this compound demonstrated low solubility (soluble in water only by 30 mg/L), chemically unstable, rapid degradation, and extensive metabolism, resulting in poor bioavailability [3,8-9]. To overcome these problems, different strategies have been implemented, such as designing new delivery systems. Nanostructured Lipid Carriers (NLC) have proven to be suitable vehicles for RSV encapsulation [7, 9-14]. NLC consists of a mixture of liquid and solid lipid, which create an imperfect crystalline matrix, providing more space between the matrix and the lipid chain, then leads to an increased the number of cavities, facilitating the accommodation of the encapsulated compound while preventing early drug release [13,14]. Therefore, RSV-NLC characterization and penetration profile studies were conducted.

Materials and Methods

Materials. RSV was purchased from Naturalin (China). Medium-chain triglyceride (Crodamol™) was a gifted sample from Croda (Singapore). Cetyl palmitate was bought from BASF (Germany). Span 20 was purchased from Brataco (Indonesia). Tween 80 was obtained from Kao Corporation (Japan). All of these chemicals were in pharmaceutical grade.

Tools. High Shear Homogenizer (T25 Ultra-Turrax IKA®), Double Beam Spectrophotometer UV-Vis (Shimadzu UV-1800), Zeta Potential and Submicron Particle Size Analyzer (Delsa™Nano), Zetasizer Nano (Malvern Panalytical), Transmission Electron Microscope (Jeol JEM-1400), pH Meter (Schott Glass Mainz, GC 824 type), Viscometer (Brookfield), Differential Scanning Calorimeter (Shimadzu 60Plus), X-Ray Diffractometer (Philips) and Fluorescence Microscope (FSX 100 Olympus).

Methods.

Preparation of RSV-NLC. Preparation of RSV-NLC was made by the high shear homogenization method. Firstly, the RSV and Crodamol™ were mixed in a glass and were put into the blend of the cetyl palmitate, Tween 80, and Span 20. Both are mixed and heated using a hot plate at 70°C. This blend was then stirred using a high-speed homogenizer at a speed of 3400rpm for 5min. On the other hand, entered a beaker glass containing acetate buffer solution (pH 4.5±0.5) at 70°C. The hot aqueous phase was gradually added with the lipid phase and homogenized at a speed of 20,000 rpm for 3 mins in five cycles. This preparation then cooled while stirring at 500 rpm for 30 mins until the best NLC system was obtained (Table 1).

Table 1. RSV-NLC Formulation (%w/w).

Composition	Formula 1	Formula 2	Formula 3	Formula 4
RSV	1	1	1	1
Cetyl pamitate	1	1	1	1
Crodamol™	4	4	4	4
Tween 80	5	6	7	8
Span 20	5	6	7	8
Acetate buffer	Until 100%			

RSV-NLC Characterization. RSV-NLC was characterized for entrapment efficiency of resveratrol in NLC system using UV-Vis Spectrophotometer. RSV-NLC was also identified for their melting temperature using Differential Scanning Calorimeter and crystal lattice using an X-ray Diffractometer. RSV-NLC is then evaluated for their physicochemical characterization such as organoleptic test, pH evaluation using pH meter, viscosity test using Viscometer, zeta potential

evaluation using a Zetasizer, particle morphology using Transmission Electron Microscope, ¹ particle size and Polydispersity Index (PI) using Particle Size Analyzer.

Penetration Profile. Penetration profile in vivo of RSV-NLC was identified using mice's (*Mus musculus*) skin profile. One hour before use, male mice were anesthetized with ketamine (50mg/kg BW) intraperitoneally. The hair on the back of the mice was shaved with a mechanical hair clipper, and the sample (with rhodamine B dye) was applied. After waiting periodically for 2, 4, and 6 hours, the mice were sacrificed by dislocation. The sample skin was cut using a cryotome and observed for ¹¹ luminescence using a fluorescence microscope with a magnification of 42X. This research was approved by the Animal Care and Use Committee (ACUC) of Airlangga University with Approval Code 738-KE.

Results and Discussion

Entrapment Efficiency. Based on (Table 2), it can see that the entrapment efficiency of RSV-NLC shows the NLC system gives a good entrapment for drugs (>80%). It can conclude that the increase in surfactant levels affects the entrapment efficiency of the NLC resveratrol statistically.

Melting Temperature Analysis. Examination with DSC aims to observe any changes in the thermogram and a shift in the melting point. The thermogram overlay (Figure 1) shows a decrease in the melting point with increasing surfactant levels. This decrease is due to the mechanism of surfactants in forming micelles that then coat the system to reduce its surface tension and interfacial parts to be modified. This phenomenon causes a decrease in the mechanical energy for dispersion [15].

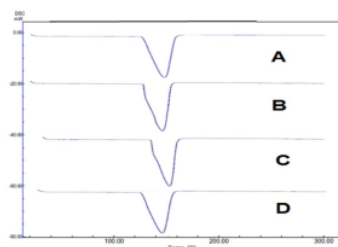


Figure 1. The results of the thermogram of the RSV-NLC (A) Formula 1; (B) Formula 2; (C) Formula 3, and (D) Formula 4.

Crystal Lattice Analysis. Based on the results of the XRD diffractogram (Figure 2), it is known that there is a decrease in peak intensity in the NLC system. These XRD results show that the NLC system is in an amorphous form. Meanwhile, in the NLC resveratrol system, two peaks appeared, which indicated an interaction in the system. It can assume that this is a metastable form of the transient resveratrol NLC system. The presence of phase separation physically proves this since the product was first made, but it becomes homogeneous after being stored for more than three days.

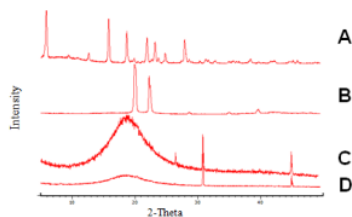


Figure 2. The results of the X-ray diffraction (A) Resveratrol; (B) Cetyl palmitate; (C) NLC system, and (D) RSV-NLC.

Organoleptic Test. The organoleptic observation showed that the NLC was yellowish, odorless, and had a little thick consistency and soft texture, as shown in (Figure 3).



3
Figure 3. RSV-NLC (A) Formula 1; (B) Formula 2; (C) Formula 3; and (D) Formula 4.

pH Evaluation. The pH value results show that the RSV-NLC has a pH in the range of 4.5, equal to the pH of the buffer used was 4.5 ± 0.5 (Table 2). The use of the pH buffer is intended so that the active ingredient remains at its pH stability. The active ingredient remains in molecular shape, and the more active ingredients penetrate the skin. In addition, pH measurements are also needed to prevent irritation and dryness of the skin. pH that is too acidic can cause irritation and sting, while a pH that is too alkaline can cause itching and dryness of the skin, so the preparation needs to be maintained in the skin pH range (4.0 – 7.0) [16].

Viscosity Test. The viscosity value shows that the addition of surfactant increases the viscosity of the preparation (Table 2). This phenomenon also proves Stoke's law that the addition of surfactant is linearly proportional to the increase in thickness. The more surfactant is added, the more friction generated by micellar aggregation will be higher until that causes an increase in viscosity.

Table 2. Physicochemical characterization of RSV-NLC.

Formula	Formula 1	Formula 2	Formula 3	Formula 4
Entrapment efficiency [%]	93.92 ± 0.22	87.92 ± 0.08	89.78 ± 0.19	91.07 ± 0.38
pH value	4.72 ± 0.08	4.62 ± 0.02	4.58 ± 0.06	4.53 ± 0.00
Viscosity [cps]	0.87 ± 0.03	0.99 ± 0.02	1.18 ± 0.03	1.50 ± 0.02
Zeta potential [mV]	-44.1 ± 0.25	-41.2 ± 0.29	-37.5 ± 0.20	-42.0 ± 0.61
Particle size [nm]	627.00 ± 101.00	584.40 ± 107.70	791.03 ± 111.03	860.70 ± 52.31
PI	0.276 ± 0.07	0.292 ± 0.02	0.287 ± 0.07	0.338 ± 0.03

Zeta Potential Evaluation. The zeta potential was found $< (-)25\text{mV}$ (Table 2). It could indicate that RSV-NLC systems had been thought to be stable colloid dispersion. The RSV-NLC systems were considered sufficient repulsive force to attain a high degree of colloidal physical stability [17].

Particle Morphology. The results of the particle morphology (Figure 4) show that both systems have spherical particles, except for Formula 2. Spherical particles can avoid coalescence between particles and friction between smaller particles. In addition, the spherical shape of the particles also facilitates the penetration of the drug substance particles into the skin. Then these spherical particles have a less flat surface. This phenomenon is probably the formation of the liquid lipid that coats the particles in the system forming a flip-flop structure.

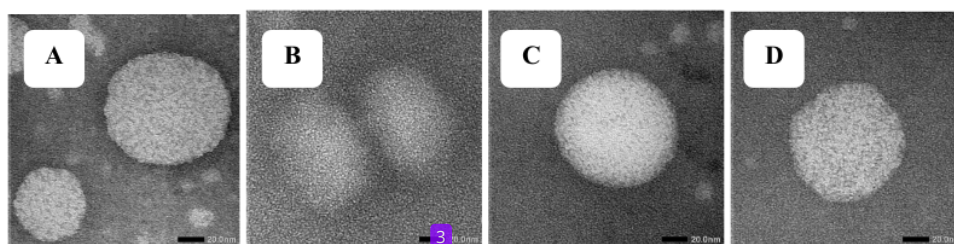


Figure 4. Particle morphology of RSV-NLC (A) formula 1; (B) formula 2; (C) formula 3; (D) formula 4 using Transmission Electron Microscope (TEM) on 20nm scale.

Particle Size and PI Measurement. In the particle measurement results, it can be seen that the addition of surfactant affects the resveratrol NLC particle size statistically (Table 2). Coalescence is possible when resveratrol is added to the NLC system. However, this particle size is still following the usual of NLC, 10-1000nm [18]. Meanwhile, the PI value is less than 0.3, which indicates a homogeneous particle size.

Penetration Profile. The penetration test was conducted to determine the depth of penetration of the skin by particles. From the results of penetration into the skin for six hours (Figure 5), it can be seen that RSV in the NLC system can increase the depth of penetration in the skin compared to RSV dissolved in a carrier oil (MCT), which is only able to penetrate the skin at the sixth hour. Based on those results, it is known that the addition of the active ingredient RSV can increase controlled drug release [19] so that it can penetrate deeper skin than resveratrol in MCT.

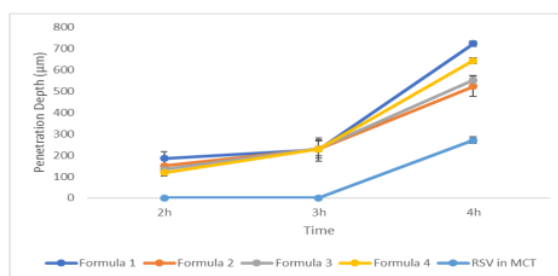


Figure 5. In vitro skin penetration study of RSV-NLC Formula 1 – 4 and RSV in Medium-chain triglyceride dispersion, using Olympus FX-100 Microscope with 42X magnification.

Conclusion

This research can conclude that the different surfactant ratios of Tween 80 and Span 20 affect the characteristics of RSV-NLC but do not affect the penetration depth of RSV in the skin statistically. The authors recommend Formula 1, as the best formula, due to its good characteristics, physicochemical properties, and skin penetration profile in vivo. This matter proved that the NLC system is one of the suitable systems as the delivery system for poorly water-soluble and weak-acid drugs.

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