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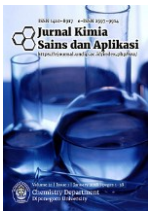

Preparation and Characterization of Nanostructured Lipid Carriers Retinol Based on Patchouli Oil (*Pogostemon cablin* Benth.): Characteristic Test and Release Profile

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Abstract

Nanostructured Lipid Carriers (NLC) were developed as a topical delivery system to overcome the limitations of retinol, such as low stability and uncontrolled release. This study used patchouli oil (PO) as a liquid lipid component to enhance the efficiency of retinol encapsulation and lipid matrix stability. The purpose of this study was to create, characterize, and test the release profile and in vitro antioxidant activity of the NLC retinol-patchouli oil (NLC-RPO) system. Optimization was performed by determining the ratio of solid lipid to liquid lipid, and the formulation was prepared using the high-shear homogenization and rapid cooling (ice bath) method. Characterizations carried out included particle size, PDI, zeta potential (ZP), morphology, entrapment efficiency (EE), FTIR, physical stability, pH, and viscosity. The release profile was tested using a Franz diffusion cell. The optimal NLC-RPO formula (1.2% cetyl alcohol, 0.8% patchouli oil, 0.3% retinol, 1% span 60, 4% tween 80) was successfully obtained and demonstrated suitable characteristics: particle size of 114.4 ± 0.42 nm, PDI 0.29 ± 0.02, ZP -32 ± 1.58 mV, EE 86.56 ± 4.24 % (n=3), and spherical morphology. The physicochemical evaluation revealed a pH of 5.59, pseudoplastic flow behavior, and good physical stability as confirmed by centrifugation testing. The NLC-RPO formulation demonstrated a modestly slower in vitro release rate at earlier time points vs free retinol under our test conditions. The antioxidant activity, tested using the DPPH method, showed that NLC-RPO had stronger antioxidant activity, with an IC_{50} of 99.59 ± 0.49 (n=3), compared to retinol and PO. Further research could conduct retinol degradation tests and in vivo penetration tests to comprehensively validate dermatological efficacy or to develop it into an anti-aging cosmetic.

1. Introduction

Skin aging is a process of progressive decline in skin function and capacity [1]. Aging can be caused by extrinsic and intrinsic factors. Extrinsic factors are those caused by environmental exposure, such as ultraviolet and infrared radiation, air pollution, and climate [2]. Intrinsic factors are caused by natural processes as we age, including hormonal changes, AGEs metabolism, immune system decline, and genetic conditions [3]. Extrinsic and intrinsic factors can trigger the formation of ROS (Reactive Oxygen Species), leading to oxidative stress in skin cells [4]. Oxidative stress can cause collagen and elastin degradation, lipid peroxidation of cell membranes, DNA damage, and activation of matrix metalloproteinases (MMPs), which ultimately cause various changes in the skin, such as thinning of the skin layer, changes in pigmentation and texture, the formation of fine wrinkles, loss of skin moisture and elasticity, and decreased skin regeneration ability [5]. Currently, skin aging is a focus of research in both dermatology and cosmetic development [6]. In the development of topical cosmetics, one

substance with anti-aging properties is retinol, a first-generation derivative of vitamin A in the retinoid family [7].

The potential of retinol as an anti-aging agent is supported by clinical evidence, which is topical application of liquid crystalline serum with retinol concentrations of 0.15% and 0.3% for 8 weeks reduced the average wrinkle depth by up to 12% and increased skin elasticity by up to 10% on average [8]. Other researchers clinically demonstrated that retinol at a concentration of 0.3% increased skin firmness by 8.0% and reduced wrinkle depth by 12.4% after 12 weeks [9]. The anti-aging potential of retinoid serum, which is a group of vitamin A derivative compounds, is also supported by *in vitro* findings, characterized by an increase in collagen synthesis by 14.6% and elastin by 16.5%, and a decrease in the secretion of collagen degradation enzymes (MMP-1) by 15.1% after exposure to UVB radiation [10].

Furthermore, the efficacy of retinoids to combat photoaging has been evaluated comparatively through a network meta-analysis in which subjects who used retinol experienced significant improvements with an odds ratio of 14.10 times for fine wrinkles and 5.49 times for skin roughness compared to subjects who only used a placebo [11]. Meanwhile, *in silico* testing also supports the potential of retinol as an anti-aging agent, as demonstrated by strong binding to the degradative protein MMP-13 with a binding affinity of -10.07 kcal/mol and MMP-9 with -8.20 kcal/mol [12].

Although retinol is known to be an effective anti-aging agent, its topical application poses physicochemical and clinical challenges. Chemically, retinol is susceptible to degradation, particularly when exposed to light and oxygen. This instability is evidenced by decreases in levels of 16 retinoid derivatives in 12 commercial products, which range from 0–80% after 6 months of storage at 25°C and 40–100% under accelerated storage conditions at 40°C [13]. In addition to stability issues, retinol also poses significant clinical challenges, particularly related to its potential for skin irritation.

Ex vivo studies have shown that retinol application increases the expression of the pro-inflammatory cytokines TNF-α by 55% and IL-1α by 57% [14]. This irritating effect is supported by the uncontrolled release pattern of retinol, up to 55% within 30 minutes (following first-order kinetics), compared to cationic nanoparticles, which managed to control the release to only 15% in the same time (following the Higuchi model) [15]. A similar rapid release pattern was also found in pure vitamin A palmitate, which showed release rates of 76.64% at the end of the third hour and 96.95% at the end of the sixth hour, compared to its nanoparticle form [16]. Therefore, to overcome the problems with retinol and control its release, it is necessary to develop a delivery system, one of which is through Nanostructured Lipid Carriers (NLC) encapsulation [17].

NLC is a colloidal matrix composed of solid lipids, liquid lipids, and emulsifiers in the size range of 50 to 500 nm [18]. NLCs are in the submicron size range (50–1.000 nm) with the main advantages of better long-term storage stability, higher active substance loading capacity, and the ability to control release behavior through the selection of appropriate lipid and surfactant compositions [19]. NLC is a drug or active ingredient carrier system that is oil-soluble (hydrophobic). The NLC system has the advantages of high encapsulation capacity, controlled release, and thermodynamic stability [17]. The solid lipid component in NLC plays a role in maintaining matrix stability through the formation of a crystal lattice [20]. Meanwhile, the liquid lipids in the NLC system form a flexible structure, thus preventing breakdown and protecting the stability of the encapsulated substances well [21]. So far, the development of NLC delivery systems has been widely researched as an alternative to overcome the weaknesses of active substances, achieve maximum delivery, control release, reduce irritation effects, and achieve certain therapeutic effects [22].

Previous research successfully produced retinol NLC using a vacuum method, with particle sizes of 200 nm, a PDI of 0.3, and a zeta potential of approximately -50 mV. Retinol encapsulated in NLC also maintained its concentration stability of approximately 90% after 4 weeks of storage at temperatures of 25, 40, and 50°C [23]. Another study on NLC was also successfully conducted with Coenzyme Q10, resulting in a particle size of 215.03 nm and an entrapment efficiency of 75.57% [24]. Furthermore, the development of NLC tretinoin, a form of retinoid that is more potent than retinol, produces spherical morphology, particle size <600 nm, polydispersity index <0.5, and zeta potential ±19 mV, as well as entrapment efficiency >80% [25]. Another study developed a tretinoin NLC system that showed controlled release reaching only 76.98% within 8 hours, compared to a tretinoin suspension that experienced rapid release of up to 98.66% within the same time [26].

In this study, an NLC formula was designed by integrating patchouli essential oil (PO) as a liquid lipid component. The potential of patchouli oil has been developed in NLC systems in several previous studies. The use of patchouli oil in an NLC Coenzyme Q10-based sleeping mask preparation has been proven to increase the physical stability of the preparation after 90 days at 20 ± 1°C and 65% relative humidity, compared to an NLC Coenzyme Q10 sleeping mask preparation without patchouli oil [27]. Other research also developed NLC patchouli oil with particle sizes of 202.53 ± 4.77 nm, a polydispersity index of 0.36 ± 0.01, a zeta potential of -22.78 ± 2.14 mV, and an entrapment efficiency of 88.89 ± 3.08% [28].

As a liquid lipid component in NLC, patchouli oil can distort the crystal structure of solid lipids, thus preventing retinol from easily escaping from the matrix [29]. Comparative research between essential oils and synthetic oils (caprylic/capric triglyceride) shows that essential oils produce smaller, more homogeneous nanoparticles with a more regular structure [30]. In addition, other studies have shown that Lavandula essential oil, when used as a liquid lipid, increases nanoparticle stability and results in a more regular chain

arrangement compared to synthetic isopropyl myristate [31]. NLCs can form an occlusive lipid film, reducing TEWL and potentially enhancing skin penetration. PO was explored as the liquid lipid component within this framework [32].

In addition, the patchoulol component also plays a role in the antioxidant activity of patchouli oil. Research has demonstrated the antioxidant activity of patchouli oil with an EC_{50} value of 19.95% [33]. Other researchers also tested the antioxidant activity of patchouli oil with an IC_{50} of 13.123 µg/mL (very strong category) [34]. Therefore, the use of patchouli oil in this study was not only to maximize the encapsulation capacity of retinol in the NLC system, but also to increase matrix stability, control retinol release, and increase the antioxidant activity of patchouli oil-based retinol NLC (NLC-RPO).

Although several studies have developed retinoids and their derivatives in NLC systems, to our knowledge, no study has specifically used PO as a liquid lipid component in the preparation of NLC systems containing retinol [23]. Previously developed formulas tend to use conventional liquid lipids. Based on the previously mentioned studies, essential oils, including PO, are able to produce nanoparticles that are smaller, more homogeneous, and have a more regular structure than synthetic oils (caprylic/capric triglycerides) [30]. Therefore, this study aims to create a patchouli oil-based retinol NLC delivery system (NLC-RPO). Its characterization is reviewed based on particle size, entrapment efficiency, and particle morphology. Evaluation of the release profile and antioxidant activity was tested *in vitro* to compare the release of retinol in NLC with free retinol, as well as to compare the antioxidant activity of the combination of retinol and patchouli oil in NLC with its single component.

This study is expected to produce appropriate NLC characteristics, a more controlled retinol release profile, and increased antioxidant activity that correlates with anti-aging capabilities. In addition, this study provides a methodological basis for other researchers to explore and develop solutions to overcome the problems of cosmetic active ingredients with similar stability issues and release limitations.

2. Experimental

2.1. Materials

The materials used in this research include: cosmetic-grade retinol powder (Joanna Biotech Chemical), patchouli oil (PT. Qiandra Aromatic Indonesia), cetyl alcohol (PT. Iniko Karya Persada), Span 60 (PT. Graha Jaya Pratama Kinerja), Tween 80 (PT. Samiraschem Indonesia), distilled water, phosphate buffer pH 7.4, technical ethanol 96% (SAE PT. Jayamas Medica Industri), methanol (Merck, Germany), DPPH (Merck, Germany), and vitamin C (CSPC Weisheng Pharmaceutical, China).

The tools used in this research include: analytical balance (Shimadzu, Japan), melting point analyzer (Stuart, UK), homogenizer (IKA Ultra-Turrax T25, Germany), ultrasonic probe (Biobase UCD-PO1, China), hotplate magnetic stirrer (SH Scientific, China), UV-Visible spectrophotometer (Genesys, USA), particle size analyzer (Malvern Zetasizer Pro / ZSU 3200, UK), fourier transform infra red (MicroLab Cary 630, USA), pH meter (Hanna Instruments, USA), viscometer (NDJ-8S, China), transmission electron microscope (TEM HT7700, Hitachi, Japan), franz diffusion cell (Permegear), and centrifuge (Laboao, China and Thermo Scientific, AS).

2.2. Determining the Ratio of Solid Lipids to Liquid Lipids

The optimal ratio between the solid and liquid lipid phases was determined using a melting-point test comparing two solid lipids: cetyl alcohol (CA) and glyceryl monostearate (GMS). Each tested ratio was prepared using a total lipid mixture weighing 2 grams. Each solid lipid, with a melting point of 58–59°C, was melted to a temperature 10°C above its melting point (68–69°C) to ensure complete melting. Patchouli oil (PO), a liquid lipid, was then added to the melted solid lipids at the ratios specified in Table 1, and the mixture was allowed to stand until it reached the desired melting point.

The solidified mixture was then transferred to a glass capillary tube, and its melting point was tested using a melting point analyzer (Stuart, UK) [35]. All melting point tests were performed in duplicate. The criteria for determining the optimal ratio were a mixture of cetyl alcohol-patchouli oil (CA-PO) or glyceryl monostearate-patchouli oil (GMS-PO) that exhibited an average melting point above 40°C. This criterion is the primary requirement for thermal stability at room temperature [36]; at the same time, NLC-RPO has the ability to gradually melt closer to body temperature. However, DSC was not performed in this study. The ratio of solid lipids to liquid lipids is shown in Table 1.

Table 1. Ratio of solid lipid and liquid lipid

Mixture	GMS (%)	CA (%)	PO (%)
1	9	0	1
2	8	0	2
3	7	0	3
4	6	0	4
5	5	0	5
6	0	9	1
7	0	8	2
8	0	7	3
9	0	6	4
10	0	5	5

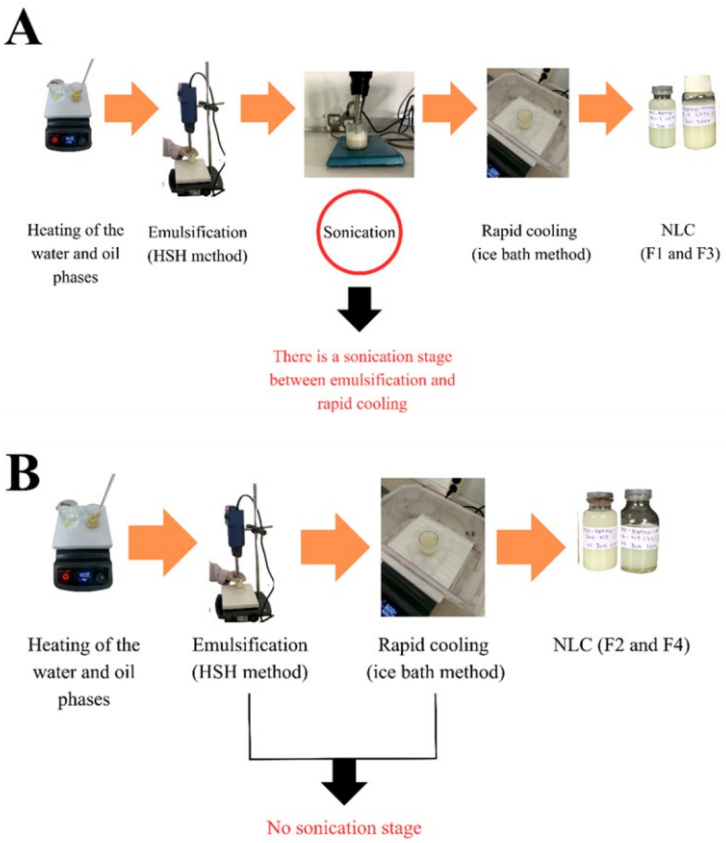


Figure 1. NLC-RPO preparation procedure (A) sonication (B) without sonication

2.3. Preparation of Nanostructured Lipid Carriers-Retinol Patchouli Oil (NLC-RPO)

The NLC-RPO was synthesized using a combination of high-shear homogenization and ultrasonication techniques. The formulations were prepared based on the optimal solid-to-liquid lipid ratios determined in the previous screening stage, specifically for mixtures 3, 4, 5, 8, 9, and 10. The oil phase, comprising solid lipids (cetyl alcohol or glyceryl monostearate), Span 60, patchouli oil, and retinol, was mixed and heated to 60°C. Concurrently, the aqueous phase was prepared by dissolving Tween 80 in distilled water and heating it to the same temperature. The aqueous phase was then added to the oil phase and homogenized at 12,000 rpm for 10 minutes.

To evaluate the effect of processing conditions, two variations were produced: with and without sonication. For the sonicated samples, an ultrasonic probe was applied for 5 minutes at an amplitude of 60% with a 10-second on/off pulse cycle [37]. These parameters were strictly controlled to prevent sample overheating. Subsequently, the mixture was transferred to an ice bath for rapid cooling and stirred at 600 rpm for 15 minutes using a magnetic stirrer to ensure solidification. In contrast, the non-sonicated variation proceeded directly to the ice bath cooling stage immediately after homogenization. The overall preparation process is illustrated in Figure 1.

The optimal NLC-RPO formulations were selected based on their physical stability during room-temperature storage. The primary criteria included the absence of phase separation, precipitation, and lipid solidification. Based on these parameters, four formulations were chosen for further characterization: GMS-S (F1) and GMS-NS (F2), representing formulations with glyceryl monostearate (GMS) at a 6:4 ratio with and without sonication, respectively; and CA-S (F3) and CA-NS (F4), representing formulations with cetyl alcohol at the same ratio. In contrast, formulations with a 7:3 ratio exhibited lipid solidification characterized by a grainy texture. Meanwhile, the 5:5 ratio led to phase separation between the aqueous and organic phases, as evidenced by the appearance of pearlescence upon agitation.

2.4. Characteristics Test (Particle Size, Polydispersity Index, and Zeta Potential)

Particle size (Z-Average), polydispersity index (PDI), and zeta potential (ZP) were analyzed using a Malvern Zetasizer instrument. The NLC-RPO tested were the four optimal formulations selected in the previous manufacturing stage (F1, F2, F3, and F4). The NLC-RPO samples were diluted 1:10 with distilled water and homogenized with a vortex to eliminate double-scattering. Z-Average and PDI were measured using the dynamic light scattering (DLS) principle, where a PDI value close to 0 indicates a uniform particle distribution, while a value close to 1 indicates a heterogeneous system. Meanwhile, ZP was measured by electrophoresis to assess electrostatic stability. The criteria for NLC to be physically stable if the ZP value is less than -30 mV or more than +30 mV [38]. All measurements were carried out in triplicate at a temperature of 25°C. The formulation exhibiting the smallest particle size, a PDI closest to 0, and a ZP < -30 mV was selected for further evaluation.

2.5. Entrapment Efficiency Test (EE)

The entrapment efficiency (EE) of retinol within the NLC-RPO matrix was determined using an indirect method. Based on the preceding characterization results (smallest particle size, PDI ≈ 0, and ZP < -30 mV), formulations F3 and F4 were selected for analysis. A 1.5 mL aliquot of the NLC-RPO sample was transferred into a centrifuge tube and centrifuged at 20,125 × g for 20 minutes to separate the lipid matrix from the unentrapped retinol. The resulting supernatant was filtered through a 0.45 µm PTFE syringe filter. Subsequently, 1 mL of the filtrate was diluted to a final volume of 5 mL with distilled water. The concentration of free retinol in the supernatant was quantified using a UV-Vis spectrophotometer at a wavelength of 325 nm, corresponding to the absorption maximum of retinol [29]. Absorbance values were converted into concentration using a previously established linear regression equation from a retinol standard curve [39]. While this method is subject to limitations regarding receptor medium and potential matrix interference, blank corrections were performed to minimize background noise. To enhance specificity, HPLC-UV with chromatographic separation is recommended for future studies, in accordance with ICH Q2(R1) guidelines. The EE percentage was calculated using Equation (1).

$$EE\ (\%) = \frac{\text{Total retinol added - Unentrapped retinol}}{\text{Total retinol added}} \times 100 \qquad (1)$$

2.6. Fourier Transform Infrared Test (FTIR)

FTIR spectroscopy was performed to identify functional groups and evaluate potential molecular interactions between retinol and the excipients within the NLC matrix. The analysis included pure retinol, patchouli oil, cetyl alcohol, Span 60, Tween 80, distilled water, a blank NLC (without retinol), and the NLC-RPO formulation exhibiting the highest EE. Solid samples were placed directly onto the ATR crystal, while liquid samples were applied in sufficient quantities to ensure adequate surface coverage. Spectra were recorded over a wavenumber range of 4000–450 cm^{-1}. The analysis focused on comparing characteristic peaks to detect shifts, broadening, or the disappearance of signals, which would indicate physical or chemical interactions between the components [40]. The resulting spectral data were processed and visualized using OriginLab software to facilitate precise peak identification and interpretation of the NLC-RPO system.

2.7. NLC-RPO Stability Test

This test was conducted to predict the long-term physical stability of NLC-RPO through accelerated mechanical stress. The sample tested was F4, which has been determined as the most optimal formula based on the EE test results. The NLC-RPO sample was placed into a centrifuge tube. The tube was then centrifuged at 3.070 × g for 30 minutes. The stability of NLC-RPO was evaluated by visually observing phase separation or sedimentation after centrifugation. The dispersion system was considered stable if there was no phase separation or other signs of physical instability. Observations were made immediately after centrifugation for a period of 30 minutes, to observe phase separation due to sudden mechanical stress, and 24 hours after centrifugation to ensure the thermodynamic stability of NLC-RPO [41]. Long-term stability ideally includes monitoring of particle size, PDI, zeta potential, and pH under standardized storage conditions.

2.8. NLC-RPO pH Test

The NLC-RPO pH test was conducted to ensure the dermatological safety of the preparation by verifying that the pH was within the appropriate physiological skin pH range (4.5–6.0) to minimize the risk of irritation, maintain the stability of pH-sensitive retinol, and support the zeta potential data results [42]. The test method was carried out by calibrating the pH meter using standard buffer solutions of pH 7.01, 4.01, and 10.01. The NLC-RPO sample was measured by inserting the pH meter electrode into the sample and allowing the reading to stabilize. The measured pH value was recorded as the result.

2.9. NLC-RPO Viscosity Test

The NLC-RPO viscosity test was conducted to ensure that product consistency was within the specification range, preventing sedimentation or separation of NLC particles during storage, and to identify rheological properties. The test method used a Brookfield rotary viscometer with spindle number 1. A total of 100 mL of NLC-RPO sample was placed into the container, then the spindle was dipped to the specified mark. Measurements were carried out at 8 rotation speeds: 0.3, 0.6, 1.5, 3, 6, 12, 30, and 60 rpm. At each rotation speed, the spindle was allowed to rotate for 1 minute to reach a stable condition. The viscosity (mPa.s) and the percent torque were recorded. The measurement process at each rotation speed was repeated in triplicate [43].

2.10. Transmission Electron Microscope Test (TEM)

Observations of the shape and morphology of NLC-RPO were conducted using an HT7700 transmission electron microscope (Hitachi, Japan). The observed sample was the optimal formula, F4. The sample was observed at 50,000× magnification. This test confirmed the shape, size, and distribution of NLC-RPO particles [37].

2.11. Release Profile Test

The NLC-RPO release test was performed using a Franz diffusion cell to compare the release profiles of retinol in the NLC system with those of free retinol. The sample tested was NLC-RPO (CA-NS). In this study, the receptor compartment was filled with a phosphate buffer solution at pH 7.4, and 0.1% Tween 80 was added to create a sink condition. The receptor compartment was maintained at 37 ± 0.5°C and stirred at 600 rpm. A cellulose acetate membrane with a pore size of 0.45 µm was soaked for 30 minutes in a phosphate buffer at pH 7.4 and placed between the donor and receptor compartments. The use of this membrane is due to its inert nature towards various compounds [44].

A total of 0.5 grams of the NLC-RPO sample and retinol solution were dropped onto the membrane

surface. Next, 1 mL of the sample was taken at certain time intervals, namely 1, 3, 5, 9, and 18 hours from the receptor compartment using a micropipette, and immediately replaced with 1 mL of phosphate buffer solution with pH 7.4. The samples were then measured using a UV-Vis Spectrophotometer at a wavelength of 325 nm [45]. The concentration of retinol in each sample was calculated based on the retinol standard calibration curve using the linear regression equation y = ax + b. The standard curve was prepared by dissolving retinol in a pH 7.4 phosphate buffer containing 0.1% Tween 80. The coefficient of determination of the standard curve was 0.9977, which indicated that the preparation method was valid. The cumulative percentage of retinol released at each time point was calculated and then plotted to produce a release profile. The kinetics of retinol release were evaluated using various mathematical models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas. The model with the highest coefficient of determination (R^2) was selected as the most appropriate for describing the release mechanism. The data were then analyzed using a one-way ANOVA test to compare differences between samples at each time interval.

2.12. Antioxidant Activity Test

The antioxidant activity of NLC-RPO was tested using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The test was conducted using retinol, patchouli oil, and NLC-RPO samples. The purpose of this test was to compare the antioxidant effectiveness of each sample, thereby supporting the potential of the NLC-RPO formulation as an anti-aging cosmetic preparation. The antioxidant activity test was performed by preparing a DPPH stock solution at 80 µg/mL in 96% ethanol. As a comparison, vitamin C solutions were prepared with concentrations of 2–10 µg/mL in distilled water. Next, retinol solutions were prepared at concentrations of 100–350 µg/mL in 96% ethanol. Patchouli oil sample solutions were prepared at concentrations of 200 to 1000 µg/mL in PA methanol. NLC-RPO sample solutions were prepared at concentrations of 100–500 µg/mL in distilled water. The test procedure was carried out by reacting the sample solution and DPPH solution (1:1). The mixture was vortexed for 20 seconds and incubated for 30 minutes at room temperature in the dark. The absorbance of the mixture was measured using a UV-Vis spectrophotometer at a wavelength of 516 nm [46]. Next, the percentage inhibition was calculated using Equation (2).

$$\%\ Inhibition = \left(\frac{A_0 - A_1}{A_0}\right) \times 100\% \qquad (2)$$

The IC_{50} value was determined by linear regression of sample concentration versus percentage inhibition ($R^2 \geq 0.97$). All antioxidant activity tests were performed three times to ensure data accuracy. The sample with the lowest IC_{50} value indicated the strongest antioxidant potential. The data were then analyzed using one-way ANOVA statistics to compare antioxidant activity between samples.

3. Results and Discussion

3.1. Determining the Ratio of Solid Lipids to Liquid Lipids

The results of melting point testing at various ratios are presented in Figure 2. The melting point decreases as the proportion of liquid lipid increases. At a ratio of 9:1 (solid lipid: liquid lipid), the mixture of GMS and CA showed the highest melting points of 68.3°C and 63.5°C, respectively. When the lipid: liquid ratio reached 5:5, the melting points of both mixtures decreased to 50.8°C for GMS and 50.3°C for CA. This decrease in melting point is due to melting-point depression, which occurs when a liquid lipid is introduced into a solid lipid matrix. This is because liquid lipid disrupts the regularity of the solid lipid crystal lattice [47]. In NLC, solid lipid remains in the α polymorph form after solidification, and liquid lipid tends to form amorphous nuclei that produce a high drug payload because the lipid matrix will crystallize in a less regular amorphous state. Mixing lipids with different chemical characteristics, such as carbon chain length and saturation level, disrupts the crystal lattice and alters crystallization, allowing the lipid matrix to accommodate a larger amount of drug and reducing the likelihood of release during storage compared to using a single lipid [48].

These test results indicated that a lipid mixture ratio with a melting point >40°C was selected to ensure the thermal stability of the NLC-RPO formula [36]. The selection of the lipid mixture ratio with a melting point >40°C in this study was based on two primary considerations: storage stability and the retinol release profile at body temperature. According to ICH guidelines, accelerated stability testing of pharmaceutical and cosmetic products is conducted at 40°C ± 2°C and 75% RH ± 5% RH to predict long-term stability. Therefore, the melting point of the lipid mixture should exceed this temperature to prevent premature melting and loss of structural integrity during storage [49].

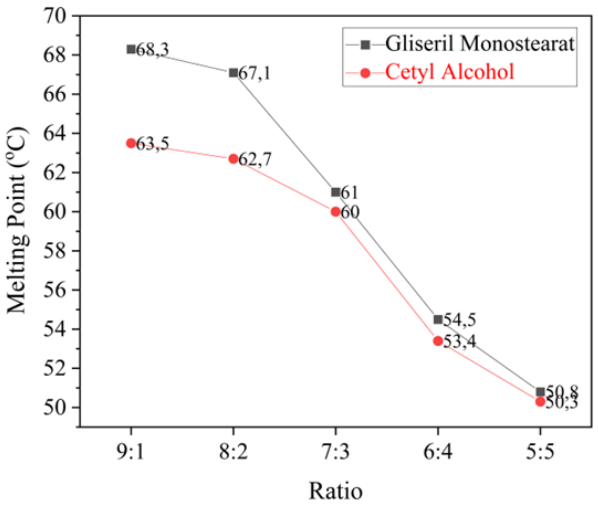
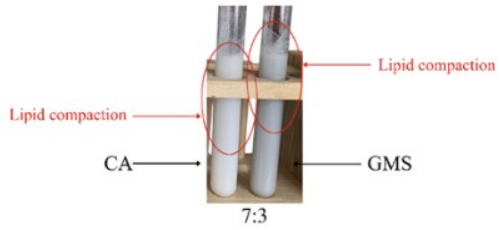
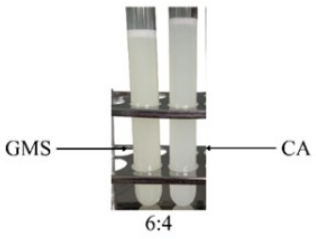
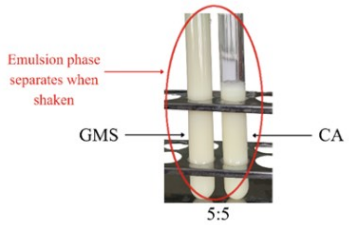


Figure 2. Melting points for mixtures of solid and liquid lipids; n = 2

Table 2. Comparison of NLC-RPO 3 ratios

Ratio	Type of Lipid	Visual	Figure
7:3	CA	Grainy texture and lipid compaction	
	GMS	Grainy texture and lipid compaction	
6:4	CA	Stable	
	GMS	Stable	
5:5	CA	Showed a pearlescent separation of the water and oil phases	
	GMS	The emulsion phase separates	

From a release perspective, a lipid-based system with an adjustable melting point allows the release of active ingredients at body temperature (37°C) for transdermal application. A melting point >40°C (ideally 40–50°C) allows the NLC matrix to undergo a partial phase transition upon contact with skin (32–34°C) and body temperature (37°C), resulting in a controlled release of the active ingredient without loss of overall structure [50]. A melting point that is too high (>60°C) can inhibit drug release at body temperature, while one that is too low (<40°C) can cause abrupt release and instability during storage. Therefore, ratios of 7:3, 6:4, and 5:5 were selected for further testing. These three ratios were chosen because they produced lower melting points than the 9:1 and 8:2 ratios. They were then prepared in the NLC system using two types of solid lipids (GMS and CA), yielding a total of 6 formulas. These formulas were evaluated for physical stability during storage at room temperature by observing signs of phase separation, precipitation, or lipid hardening.

The evaluation results showed that only a 6:4 ratio of the two types of solid lipids maintained physical stability, while a 7:3 ratio resulted in a grainy texture, and a 5:5 ratio showed a pearlescent separation of the water and oil phases, as shown in Table 2. The stability of the 6:4 ratio can be explained by the optimal balance between solid and liquid lipids in the system. This ratio produces a sufficient proportion of liquid lipids to effectively distort the solid lipid crystal lattice, creating sufficient void space for retinol encapsulation, while minimizing the release of active substances during long-term storage [51]. These results are consistent with a study by Esadini *et al.* [52], which also found the 6:4 ratio to be the most stable in linestrenol NLC formulations. Conversely, a 7:3 ratio with an excess of solid lipids tends to form a more regular, rigid crystal structure, making it more susceptible to the release of active substances due to polymorphic transformation during storage [43]. Meanwhile, a 5:5 ratio with equal proportions of liquid and solid lipids produces a matrix that tends to be soft, making particles susceptible to agglomeration and sedimentation due to the lack of structural rigidity [51]. Therefore, in this study, the 6:4 ratio was determined as the optimal formula for the NLC-RPO.

3.2. Preparation of Nanostructured Lipid Carriers-Retinol Patchouli Oil (NLC-RPO)

The NLC system designed for retinol encapsulation in this study used a combination of solid and liquid lipids from patchouli oil. The solid lipids serve as the structural framework of the nanoparticle matrix, providing physical stability and protecting retinol from degradation factors such as light and oxygen during storage [20]. Meanwhile, patchouli oil contributes to the formation of a more flexible, amorphous (irregular) lipid structure [21]. Because retinol and patchouli oil are lipophilic, they exhibit compatibility, allowing retinol to dissolve within the matrix. This amorphous structure effectively increases the retinol's loading capacity and serves as an internal reservoir [32]. This reservoir is essential for

regulating and slowing the diffusion of retinol, resulting in a controlled, prolonged release that is crucial for enhancing anti-aging effectiveness while minimizing potential irritation [53]. The optimal NLC-RPO formulas were selected at a ratio of 6:4, namely F1, F2, F3, and F4, with both sonicated and non-sonicated treatments. The four formulas were evaluated based on particle size, polydispersity index, and zeta potential to determine the type of solid lipid and treatment capable of producing the smallest particle size, a PDI value close to 0, and a zeta potential > +30 mV or < -30 mV. Based on the research conducted, the NLC-RPO formulas are shown in Table 3.

The success of the developed NLC-RPO formulation was assessed not only based on the appropriate characteristics in terms of nanoparticle size and other physicochemical characteristics, but also on the good physical stability of the nanoemulsion [24]. Achieving physical stability results from the appropriate composition design of the NLC-RPO formulation, especially the optimal lipid ratio and surfactant composition. All NLC-RPO formulas made in this study produced homogeneous yellowish-white results. NLC-RPO using GMS lipids was more opaque, and CA was more transparent, had a strong patchouli oil aroma, and a liquid consistency, as shown in Figure 3.

3.3. Characteristic Tests (Particle Size, Polydispersity Index, and Zeta Potential)

Particle size, polydispersity index, and zeta potential tests were performed to confirm that NLC-RPO was successfully formulated at the nanoscale, had a uniform size distribution, and exhibited electrostatic stability, preventing agglomeration. Data from the NLC-RPO characteristic test results are shown in Table 4. Based on the results of this research, the particle size of F1-F4 is below 200 nm, with the smallest size in F4 being 114.4 ± 0.42 nm. However, this study found that the NLC-RPO formulas not sonicated (F2 and F4) produced smaller particles than those sonicated (F1 and F3).

The phenomenon of increasing size after sonication in this study was interpreted as local heating triggered by ultrasonic energy, causing re-melting of the lipid matrix, and ultimately triggering agglomeration [33]. However, using the nonparametric Kruskal-Wallis test followed by the Dunn test, the difference between the sonicated and non-sonicated formulas was not significant ($p > 0.05$). However, significant differences were only found in F1 and F4 ($p < 0.05$). This means that the use of different types of solid lipids has a greater effect on NLC particle size than the treatment itself. The optimal size indicates CA's superiority over GMS in this study. In general, CA with a chain number (C16) tends to produce smaller particles than more complex lipids such as GMS with a chain number (C18). The structure of GMS, which is a monoglyceride and has high crystallinity, tends to form a more regular and larger crystal structure during cooling, resulting in a larger NLC particle size [54]. In contrast, CA has a simpler matrix-forming mechanism, so it can reach a smaller nanoscale. This particle size below 200 nm not only ensures system stability but also complies with the size criteria considered optimal for NLC delivery systems [55].

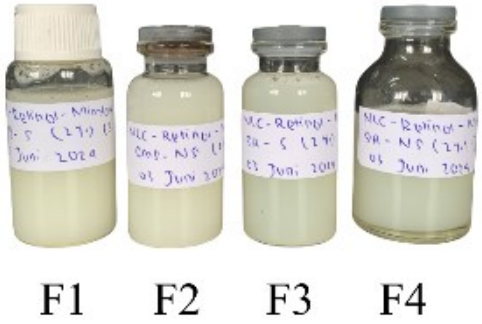


Figure 3. NLC-RPO

Table 3. NLC-RPO formula

Ingredient	Function	F1 (%) (GMS-S)	F2 (%) (GMS-NS)	F3 (%) (CA-S)	F4 (%) (CA-NS)
Glyceryl monostearate	Solid lipid	1.2	1.2	-	-
Cetyl alcohol	Solid lipid	-	-	1.2	1.2
Patchouli oil	Liquid lipid	0.8	0.8	0.8	0.8
Span 60	Co-surfactant	1	1	1	1
Retinol	Active ingredients	0.3	0.3	0.3	0.3
Tween 80	Surfactant	4	4	4	4
Distilled water	Solvent	ad 100	ad 100	ad 100	ad 100

Note: GMS-S = Glyceryl Monostearate with Sonication; GMS-NS = Glyceryl Monostearate without Sonication; CA-S = Cetyl Alcohol with Sonication; CA-NS = Cetyl Alcohol without Sonication.

Table 4. NLC-RPO characteristic

NLC sample	Particle size (nm)	Polydispersity Index	Zeta Potential (mV)	Treatment
F1	186.33 ± 5.86	0.57 ± 0.04	-27.31 ± 1.09	Sonication
F2	178.27 ± 0.06	0.50 ± 0.01	-25.43 ± 0.92	Non sonication
F3	116.27 ± 0.12	0.30 ± 0.02	-27.25 ± 1.10	Sonication
F4	114.47 ± 0.42	0.29 ± 0.02	-32.55 ± 1.58	Non sonication

Note: The results are presented as mean ± SD (n=3).

Furthermore, the polydispersity index (PDI) is a parameter for evaluating the homogeneity of dispersion, with values ranging from 0 to 1 [56]. PDI values between 0.1 and 0.3 are often considered reasonable for many drug delivery system applications [49], while PDI < 0.1 indicates very high monodispersity and PDI > 0.7 indicates a very heterogeneous or polydisperse size distribution according to the ISO 22412:2017 standard [57]. Test results show that all NLC-RPO formulas have PDI values > 0.2, ranging from 0.29 to 0.57 (Table 3). Although the PDI values of all formulas were above 0.2, F4 was still within the acceptable range (<0.3) for lipid nanoparticle-based drug delivery systems, even approaching the upper limit of the narrow distribution category according to the ISO standard [57].

Based on the Kruskal–Wallis test, PDI values across all formulas were not significantly different ($p > 0.05$), indicating that lipid matrix type and sonication do not affect NLC-RPO particle size homogeneity. However, the diversity of PDI values obtained can be explained by several factors. First, the differences in crystallization characteristics between GMS and CA as solid lipids. Variations in lipid emulsification and crystallization processes can result in different size distributions depending on the physicochemical properties of the lipid used [29]. Second, sonication may also introduce excessive shear, leading to droplet disruption, increased polydispersity, and potential degradation of volatile components [49]. Third, the complexity of the interactions of multiple components in a system containing solid lipids, essential oils, retinol, and surfactants. Mixing lipids with different chemical characteristics creates crystal lattice disruption and alters crystallization [49]. Variations in the distribution of these components within the lipid matrix result in populations of particles of varying sizes, especially when nucleation and crystal growth rates differ during cooling. This diversity of PDI results from complex interactions among lipid types, alternative energy sources, and differences in crystallization kinetics across formulas.

Furthermore, the zeta potential measures the electrical charge on the particle surface in a dispersion, where the magnitude of the zeta potential value (positive or negative) reflects the interparticle repulsion that prevents aggregation or coalescence [54]. In general, a colloidal dispersion is considered physically stable if the absolute value of the zeta potential is less than -30 mV or more than +30 mV [38]. Based on Dunn's further test of the zeta potential values, a significant difference was found only between F2 and F4 ($p < 0.05$). This indicates that the lipid matrix type has a stronger influence on particle surface charge than sonication energy.

The results of this study showed that F4 had a zeta potential value of -32.55 mV. The other formulas showed values of -25.43 to -27.31 mV, indicating moderate stability but below the optimal threshold. The superior zeta potential of F4 may be due to the structural characteristics of CA, a long-chain aliphatic alcohol, which provides greater flexibility in forming lipid matrices than GMS, which has ester groups. The molecular structure of solid lipids affects the orientation of surfactants at the interface and the formation of an electrical double layer [47]. CA with a simpler structure (single hydroxyl group) facilitates a more regular orientation of surfactants on the particle surface and produces a more effective stabilization layer than GMS, which has an ester group that can interact in a complex with the polar head of the surfactant.

In addition, in all formulas, the zeta potential is negative; this can be associated with the pH of the NLC, which is 5.59. Ionization of lipid component groups can produce a negative charge on the NLC in a relatively acidic environment. GMS undergoes hydrolysis to produce stearic acid (pKa ~4.85), which ionizes to COO^- at pH 5.59. CA, despite having a high bulk pKa (~15-16), undergoes partial ionization at the water-lipid interface due to a decrease in the apparent pKa [58] to produce $R\text{-}O^-$. Patchouli oil containing patchoulol can undergo oxidation during the heating process to produce carboxylic acid (pKa ~4.8–5.0) [47], which ionizes to COO^- at pH 5.59 as the main source of negative charge. pH 5.59 is above the pKa of carboxylic acid, facilitating partial ionization, thus producing a negative surface charge.

3.4. Entrapment Efficiency Test (EE)

The EE test is an important parameter that indicates the percentage of retinol successfully trapped in the NLC matrix. The resulting EE percentage represents the amount of retinol trapped in the NLC matrix, while the remainder is considered to be outside the matrix as untrapped free retinol. The test results showed that F3 had an EE percentage of 83.16 ± 0.87% while F4 had an EE percentage of 86.56 ± 4.24%. Based on the Two-Sample t-test, the obtained value indicated that there was no significant difference in entrapment efficiency between F3 and F4 ($p > 0.05$). This indicates that sonication did not significantly change the retinol entrapment capacity in the NLC matrix. Retinol entrapment in the NLC matrix occurs through the distribution of lipophilic molecules in the empty spaces (imperfections) formed due to the disruption of the crystal lattice by the liquid lipid [48]. As F3 and F4 share the same composition, their lattice structure and available space for retinol are comparable. Although sonication reduces particle size and improves dispersion, it does not alter the matrix's intrinsic capacity to retain retinol [49]. Therefore, the insignificant difference in %EE between F3 and F4 confirms that encapsulation efficiency is determined by the composition and structure of the lipid matrix, not by the energy method used in the preparation.

3.5. Fourier Transform Infrared Test (FTIR)

FTIR testing was performed to identify the presence of retinol in the NLC matrix and to evaluate the chemical compatibility between retinol and the excipients in the formula (cetyl alcohol, patchouli oil, Span 80, and Tween 80). The FTIR spectrum of pure retinol showed characteristic absorption bands, including wavenumbers 3300 cm^{-1} (alcohol O-H stretching), 2900 cm^{-1} (aliphatic C-H stretching), and 1650 cm^{-1} (conjugated C=C stretching) [37]. These bands reflect the specific chemical structure of the retinol molecule, which has a terminal hydroxyl group and a conjugated polyene system.

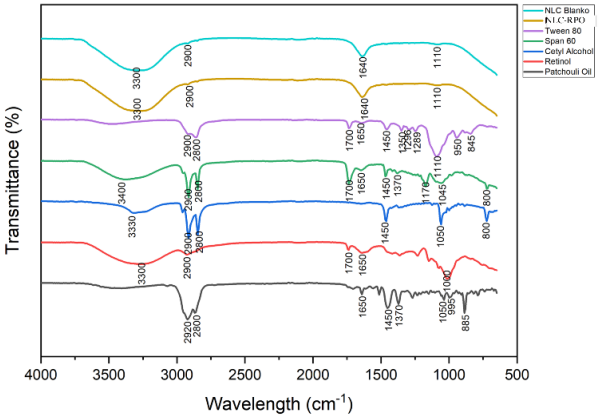


Figure 4. FTIR spectra of NLC-RPO

The NLC-RPO spectrum showed that all characteristic absorption bands of retinol were still detected, but with reduced intensities and shifted wavenumbers. The retinol O-H band at 3300 cm^{-1} is shifted or masked by the absorption of hydroxyl groups from excipients, particularly cetyl alcohol, which also has a terminal –OH group. The decrease in the intensity of the characteristic absorption band of the active substance in the NLC spectrum indicates that the active substance molecules have been molecularly distributed within the lipid matrix, thus weakening their absorption effect due to physical interactions with the lipid components [29]. This phenomenon is not an indication of degradation or a chemical reaction, but rather evidence of retinol encapsulation within the NLC matrix. Absorption bands from the lipid matrix, such as the aliphatic C-H stretching bands of cetyl alcohol (2900 cm^{-1} and 2800 cm^{-1}), are also dominant in the NLC spectrum and overlap with the retinol C-H band at the same wavenumber, which is a common phenomenon in lipid-based systems.

Furthermore, significant changes were detected in the fingerprint region (1500–500 cm^{-1}). The spectrum of pure retinol showed a characteristic band at ~1000 cm^{-1}, which originates from the C-O stretching or plane bending of the retinol alcohol group. However, in the NLC-RPO spectrum, a more dominant band appeared at 1110 cm^{-1}, characteristic of Tween 80, namely the antisymmetric C-O-C stretching of the polyoxyethylene chain. The dominance of the band at 1110 cm^{-1} in NLC-RPO indicates that hydrogen bonds form between tween 80 and retinol, thus masking the signal of retinol encapsulated in the lipid core [48].

The absence of significant new bands or the disappearance of the main characteristic band of retinol in the NLC-RPO spectrum indicates that no covalent chemical interaction occurs between retinol and the NLC components. Chemical compatibility in lipid nanoparticle systems can be confirmed by FTIR, which evaluates whether the characteristic absorption bands of the active substance remain unchanged, without the formation of new bands or drastic shifts indicative of a chemical reaction [49]. In other words, retinol and the NLC matrix are chemically compatible, and the entrapment mechanism is physical entrapment, not the formation of new compounds. These results confirm that NLC-RPO is a stable carrier system for retinol, in which retinol is encapsulated in the lipid matrix without degradation or chemical structural modification.

Furthermore, a small shift or broadening in the O-H band (3300 cm^{-1}) can be explained by the formation of hydrogen bonds between the hydroxyl group of retinols and the hydroxyl group of cetyl alcohol or with the ether oxygen of Tween 80. The formation of intermolecular hydrogen bonds in lipid nanoparticle systems can cause a shift in the O-H band to lower wavenumbers (red shift) or a broadening of the band due to varying strengths of the hydrogen bonds formed [59]. This non-covalent interaction is beneficial because it helps stabilize retinol in the lipid matrix without damaging its molecular structure, thereby maintaining its biological activity.

3.6. NLC-RPO Stability Test

The centrifugation stability test is a thermal stress method (accelerated stability test) that is used to predict the long-term physical stability of NLC systems in a short time [60]. In this test, the NLC-RPO formula was subjected to centrifugal force of 3.070 × g for 30 minutes, which is significantly greater than the usual gravitational force and is expected to accelerate potential instability phenomena such as creaming, flocculation, or sedimentation. The test results showed that the NLC-RPO formula remained stable (no phase separation, creaming, or sedimentation) after 30 minutes of centrifugation and 24 hours of room-temperature storage, as shown in Figure 5. These results confirm that this dispersion system has excellent physical stability.

The observed stability can be attributed to a zeta potential of -32.54 mV, indicating strong electrostatic repulsion between particles. This repulsive force effectively prevents nanoparticles from approaching one another and from aggregating, even under centrifugal forces. Another factor is the combination of non-ionic surfactants (Span 60 and Tween 80), which produce steric effects on the particle surface [61]. The thick surfactant layer creates a physical barrier that synergistically supports electrostatic stability, ensuring the NLC matrix remains homogeneously dispersed and stable against centrifugal forces. The stability of the NLC in the centrifugation test demonstrates that the resulting NLC matrix is robust and resistant to physical instability during manufacturing or storage.

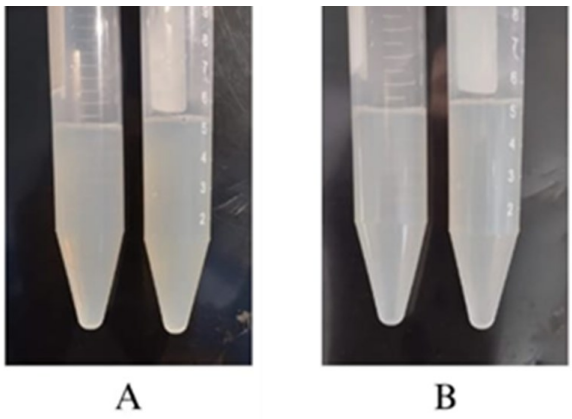


Figure 5. Stability test of NLC-RPO (A) before centrifugation; (B) after centrifugation

3.7. NLC-RPO pH Test

The pH testing of NLC-RPO is a crucial parameter for verifying the safety of topical application and the stability of the carrier system. The ideal pH range for an NLC system typically falls between 5.0 and 6.5. Test results showed a pH of 5.59 for NLC-RPO, as measured with a pH meter. This value is highly relevant because it is close to the natural pH of human skin's acid mantle, which is 4.5 to 6.0, thus ensuring dermatological tolerance, minimizing potential irritation, and preventing disruption of the skin's barrier function [1]. Furthermore, pH significantly influences the chemical stability of retinol, which is known to be highly susceptible to degradation, especially at alkaline conditions [23]. This pH value also exhibits a strong correlation with the zeta potential [62]. Therefore, the NLC-RPO pH data confirms the formulation's success in terms of topical safety and delivery system stability.

3.8. NLC-RPO Viscosity Test

Viscosity testing of NLC-RPO was conducted to ensure consistency in preparation and to assess its ability to prevent sedimentation or particle separation during storage. Viscosity test results in Figure 6 showed a consistent decrease with increasing shear rate. This pattern confirmed that the NLC system exhibited pseudoplastic (shear-thinning) flow behavior [63]. Pseudoplastic properties at low shear rates, such as during storage, result in a relatively high viscosity of the preparation, hindering particle movement. This high viscosity at rest effectively inhibits sedimentation and prevents NLC particle aggregation, thereby ensuring long-term physical stability [64]. In addition to its stability role, shear-thinning properties are ideal rheological properties for topical preparations, as viscosity decreases with increasing shear stress, providing comfort during application [65].

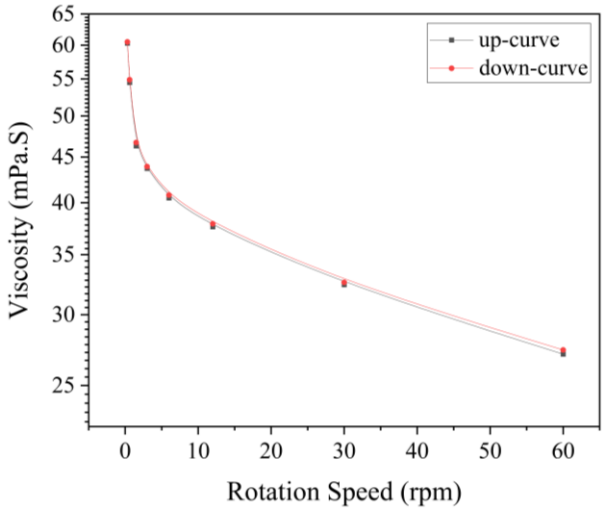


Figure 6. NLC-RPO rheology

3.9. Transmission Electron Microscope Test (TEM)

TEM was performed to visualize the morphology, shape, and size of NLC-RPO particles, thus providing visual confirmation of the DLS results. The TEM micrograph results in Figure 7 confirm that the NLC-RPO particles have a homogeneously dispersed spherical shape without significant aggregation [66]. This shape is produced during the accelerated homogenization process, driven by the decrease in surface tension due to the presence of surfactants. The formed spherical particles are then stabilized and locked by a rapid cooling process, where minimal interfacial energy facilitates the formation of a dense spherical bilayer [67]. TEM visualization also qualitatively indicates that the particle size is in the nanoscale, which supports the quantitative results from DLS. However, the DLS hydrodynamic size is usually larger than the TEM dry diameter.

3.10. Release Profile Test

In vitro release profiles using Franz diffusion cells showed that free retinol and NLC-RPO had relatively similar cumulative release patterns, with cumulative release of free retinol reaching 61.81% and NLC-RPO reaching 57.25% in 18 hours (Figure 8). Based on statistical analysis, there was no significant difference between the total cumulative release of the two formulations ($p > 0.05$). However, an important difference lies in the release profile, especially over time intervals. Retinol, as a lipophilic molecule (log P ~5.7), facilitates rapid permeation through the lipid barrier, and this rapid release can cause high local concentrations in the skin that trigger irritation, redness, and desquamation [26].

In this study, pure retinol showed a faster release rate at time intervals 1, 3, 9, and 18. This indicates that free retinol tends to undergo burst release, which has the potential to cause skin irritation upon topical application. In contrast, NLC-RPO exhibited a slower release profile over the same time interval, with a lower percent release compared to pure retinol. The encapsulation mechanism in NLC results in the majority of retinol molecules being entrapped within the lipid matrix, which acts as a physical barrier, ensuring gradual release from the lipid core to the particle surface and then into the acceptor medium [48]. The release of retinol from the nanocarrier system allows the skin to gradually adapt to retinol exposure, reducing the acute inflammatory response that often occurs with the sudden application of conventional retinol at high concentrations [49].

However, these release profile results are also attributed to limitations in the *in vitro* assay. The limited sensitivity of the UV-Vis spectrophotometry method used in this study may have contributed to the insignificant results. Certain limitations of this method compared to HPLC can also be interpreted as a factor [29]. Furthermore, synthetic membranes do not replicate the complexity of the stratum corneum, with its lipid lamellar structure and skin appendages [68]. Although the cumulative release percentage did not differ significantly, the slow-release profile of NLC-RPO over time is important for safety and skin tolerability.

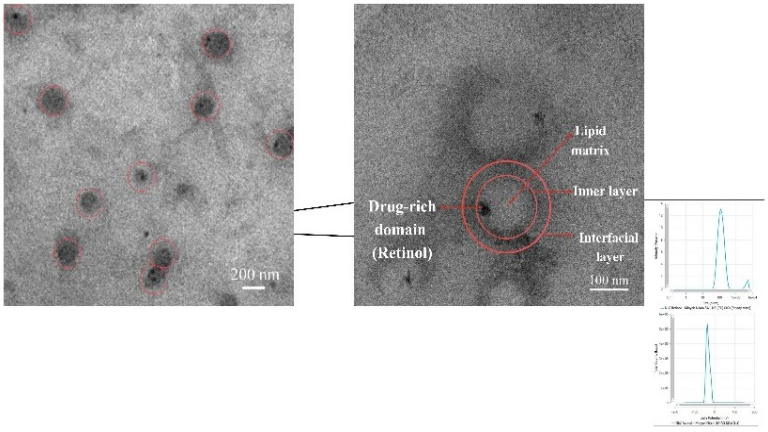


Figure 7. NLC-RPO Morphology

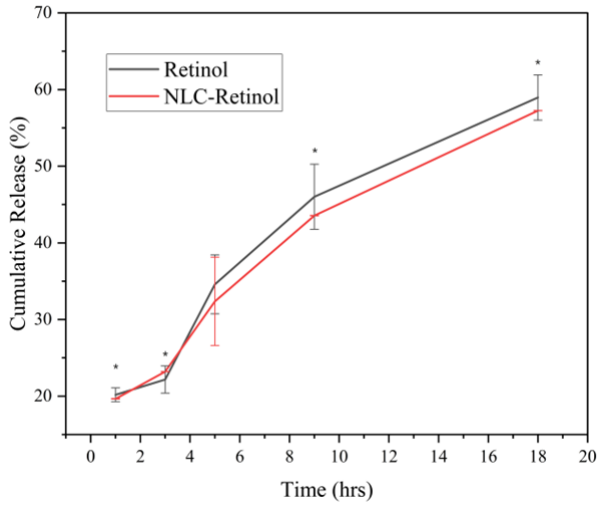


Figure 8. Release rate of retinol and NLC-RPO; n = 2; mean ± SD

Furthermore, the release profiles of pure retinol and NLC-RPO were standardized by determining release kinetics (0 order, 1st order, Higuchi, and Korsmeyer–Peppas). Based on the highest R^2 values in Table 5, the release of pure retinol followed a 1st-order kinetic model, whereas NLC-RPO followed the Higuchi model. 1st order describes an exponentially decreasing concentration-dependent release and is common in systems without a matrix barrier [59]. The Higuchi model ($Q_t = K_H \times t^{0.5}$) indicates Fickian diffusion through a solid matrix, where retinol diffuses from the lipid core of the NLC to the surface [69].

Table 5. Release kinetics

Sample	R^2	Release kinetics
Retinol	0.8727	0 Order
	0.9304	1st Order
	0.9163	Higuchi
	0.8391	Korsmeyer–Peppas
NLC-RPO	0.9609	0 Order
	0.9791	1st Order
	0.9814	Higuchi
	0.9495	Korsmeyer–Peppas

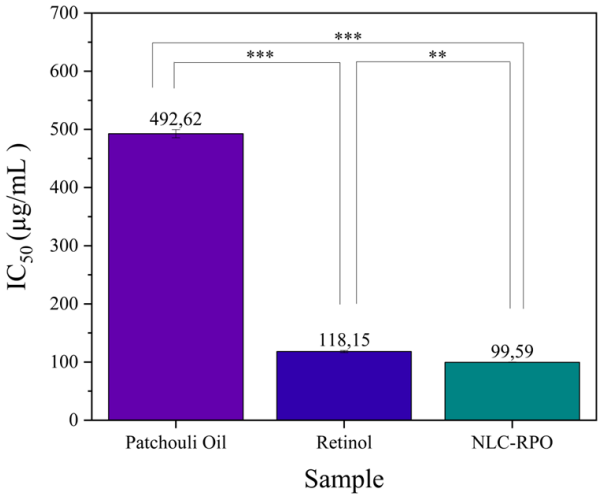


Figure 9. Antioxidant activity of retinol, patchouli oil and NLC-RPO; n = 3; one-way ANOVA and Tukey's test; ** = $p < 0.01$; *** = $p < 0.001$

3.11. Antioxidant Activity Test

The DPPH radical scavenging test showed significant differences in antioxidant activity between samples (Figure 9). Patchouli oil showed weak antioxidant activity IC_{50} = 492.62 ± 6.95 µg/mL), retinol showed moderate antioxidant activity IC_{50} = 118.15 ± 1.87 µg/mL), and NLC-RPO showed strong antioxidant activity IC_{50} = 99.59 ± 0.49 µg/mL). Statistically tested using one-way ANOVA and continued with Tukey's test, the antioxidant activity between NLC-RPO was significantly different compared with patchouli oil ($p < 0.001$) and retinol ($p < 0.01$). Patchouli oil contains sesquiterpenes such as patchoulol, which contribute to antioxidant activity through hydrogen donation [70] but sesquiterpene alcohols have moderate antioxidant activity compared to phenolic compounds [71]. Retinol, on the other hand, exhibits antioxidant activity through a conjugated polyene system that stabilizes free radicals [72], through hydrogen donation and singlet oxygen quenching mechanisms [73].

The stronger antioxidant activity of NLC-RPO may be interpreted by a synergistic effect between retinol and patchouli oil sesquiterpenes, with different mechanisms of action, resulting in a total activity that exceeds the sum of the individual components [74]. In addition, encapsulation protects retinol from oxidative

degradation and maintains antioxidant activity longer [75]. Nanoparticles with high surface area also increase contact with DPPH radicals, then facilitate a more efficient De Gruyter-Brill scavenging reaction [76]. The high antioxidant activity of NLC-RPO correlates with topical anti-aging activity because oxidative stress is a major factor in skin aging [77].

4. Conclusion

This study successfully developed a Nanostructured Lipid Carriers (NLC) system containing retinol and patchouli oil (NLC-RPO). The optimal formula obtained contained 1.2% cetyl alcohol, 0.8% patchouli oil, 0.3% retinol, 1% Span 60, and 4% Tween 80. This formula showed ideal characteristics for the NLC system, as evidenced by homogeneity, a particle size of 114.4 ± 0.42 nm, PDI of 0.29 ± 0.02, ZP of -32 ± 1.58 mV, EE of 86.56 ± 4.24% (n=3), and spherical morphology. The combination of solid and liquid lipids, along with optimal surfactants and cosurfactants, successfully provided physical stability to the preparation and showed a slightly slower in vitro release at early time points compared to free retinol under our test conditions. Furthermore, NLC-RPO showed strong antioxidant activity, with an IC_{50} of 99.59 ± 0.49, compared with retinol ($p < 0.01$) and patchouli oil ($p < 0.001$). This means that in the NLC system created, the synergy between retinol and patchouli oil can increase antioxidant activity. As a recommendation for further research, it is recommended to conduct penetration tests using animal skin, conduct *in vivo* anti-aging testing, or continue developing anti-aging cosmetic preparations. This study can also serve as a reference in solving problems with other cosmetic active ingredients that use nanoparticle systems.

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