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Original Research Article

Innovative Formulation of Solid Lipid Nanoparticle Loaded with Carrot Seed Essential Oil for Potential Antioxidant Activity and Sun Protection

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ABSTRACT

The discovery of natural sun protection and antioxidant agents is vital, but the low bioavailability of these ingredients often limits their use. This study aimed to formulate and characterize a serum containing SLN-CSEO-APs (solid lipid nanoparticle-carrot seed essential oil) with anti-aging and photoprotective properties to evaluate its antioxidant and sun protection effects. SLN was prepared using hot homogenization, heating lipids above their melting point, and dissolving the emulsifier in water. The physical characteristics, stability, antioxidant activity, and sun protection efficacy of the serum were evaluated. Antioxidant activity was assessed using DPPH and ABTS methods, while sun protection was measured by ultraviolet B (UVB) and ultraviolet A (UVA) protection. SLN-CSEO-APs serum showed a spherical morphology with a particle size of 257.12 nm ± 28.82, a polydispersity index of 0.50 PDI ± 0.00, and a zeta potential of -23.19 mV ± 0.81. The serum hydrogel had a pH within the physiological range for skin, with optimal viscosity, spreadability, and adhesion. Over time, serum stability decreased, as indicated by the reduction in all parameters. The serum demonstrated strong antioxidant potential, with IC_{50} values for ABTS and the positive control closely matched. Antioxidant activity was moderate, with values of 0.157 mg AAE/g dw for DPPH and 0.644 mg TE/g dw for ABTS. UVB protection was medium (13.549 ± 0.827), and UVA showed PA++ (4.202 ± 0.040). SLN-CSEO-APs serum provides significant antioxidant and sun protection, making it a promising skincare ingredient.

Keywords: Solid lipid nanoparticle, Carrot seed essential oil, Antioxidant activity, Sun protection

Introduction

Sun protection and antioxidant are essential factors in preventing skin damage caused by ultraviolet (UV) radiation and reducing skin cancer risks. Antioxidant functions by neutralizing free radicals generated during UV exposure, while sunscreen protects through the absorption or reflection of UV radiation.[1-2] Common strategies for sun protection and antioxidant supplementation include the application of antioxidant-enriched moisturizing creams and the consumption of antioxidant such as vitamins C, D, and E, epigallocatechin-3-gallate, and morin.[3] Previous reports have shown that synthetic antioxidant may trigger adverse skin reactions, including disorders and allergic responses, when exposed to sunlight. Furthermore, sunscreen predominantly formulated with synthetic ingredients commonly fail to provide complete protection against UV exposure.[4-5] Carrot Seed (*Daucus carota* L.) Essential Oil (CSEO) is derived from the seeds of carrot plant. Evidence suggests that CSEO offers excellent protective effects against UV radiation and oxidative stress, in addition to showing antibacterial activity. The presence of natural antioxidant, particularly beta-carotene and vitamin E, contributes significantly to these protective functions.[6-7]

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CSEO represents a potential alternative to mitigate the adverse effects and environmental concerns associated with synthetic sunscreen agents, considering the increasing regulatory restrictions on synthetic antioxidant due to toxicity and health risks. Despite the advantages, the direct application of essential oil or the incorporation into conventional cosmetic formulations presents significant limitations, specifically regarding the penetration through the stratum corneum, which is the outermost layer of the skin.[8-9] This layer consists of densely packed keratinized cells embedded in a rigid lipid matrix, formng a barrier that restricts the effective permeation of lipophilic essential oil molecules.[10-11] Consequently, only a small fraction of active compounds may reach deeper skin layers, limiting the therapeutic efficacy. The direct use of essential oil can potentially induce irritation or allergic responses because of the high concentration of bioactive components. The development of advanced delivery systems is required to improve the penetration efficiency of essential oil and minimize adverse effects.

Nanoparticle-based technology has been developed as a promising method across multiple fields, including cosmetics, due to the ability to enhance product stability, bioavailability, and therapeutic efficacy. Nanoparticles confer several advantages, such as the prevention of transepidermal water loss, improved dermal absorption, enhanced penetration of active compounds, and controlled release of encapsulated agents.[12] Among these, solid lipid nanoparticle (SLN) has gained particular attention as versatile carriers for pharmaceutical and cosmetic formulations.[13] SLN represents advanced drug delivery systems with the capacity to encapsulate diverse therapeutic molecules, characterized by targeted delivery, controlled release profiles, minimal immunogenicity, and favorable patient compliance.

This offers various practical advantages such as scalability, the elimination of organic solvents during production, relative cost-effectiveness, and ease of manufacturing, compared to other nanoparticle systems.[14] SLN is generally safer and better tolerated than polymer-based nanodispersion systems, as certain polymers may present toxicological concerns.[15] The safety and environmental challenges associated with synthetic sunscreen agents, combined with the restricted use of synthetic antioxidant due to toxicity, have prompted an urgent need for alternative strategies. These constraints limit the availability of safe and effective active ingredients for sun protection, presenting a significant challenge to the cosmetic and pharmaceutical industries. The discovery and development of natural agents with sun-protective and antioxidant potential have become increasingly important, as plants are currently considered a promising source of beneficial bioactive compounds. Therefore, this study aims to formulate SLN-CSEO-APs (Solid Lipid Nanoparticle containing Carrot Seed Essential Oil as a Potential Anti-aging and Sun Protection Strategy). SLN-CSEO-APs represent a controlled small-molecule therapeutic and protective system with high sensitivity and low toxicity (Figure 1). Development strategies of this nature are intended to improve existing formulations and introduce innovative products or enhance the utility of products in existing markets. The development efforts are grounded in consumer demand while simultaneously leveraging local natural resources to strengthen national competitiveness.

Materials and Methods

Materials

Carrot seed oil (YOUNG LIVING® Essential Oils) was purchased on March 12, 2024 from an authorized distributor, Oiljoy, located in West Jakarta, Indonesia. According to the manufacturer's information, the oil was produced by steam distillation of dried seeds of carrot. Stearic acid, ethanol, lactose, PEG 400, propylene glycol, triethanolamine, and Tween 20 (analytical grade, Merck, Darmstadt, Germany) were used in this study, and distilled water was sourced from PT. Brataco, Indonesia. Carbopol 940, methylparaben, and propylparaben were obtained from Sigma-Aldrich, a company based in St. Louis, Missouri, which supplies chemicals, reagents, and equipment for biotechnology, pharmaceutical, and chemical studies.

Preparation of SLN-CSEO-APs

The most straightforward method for the preparation of SLN is hot homogenization, which includes dissolving the active pharmaceutical ingredient in a melted lipid at a temperature above the melting point. The resulting lipid solution is subsequently mixed with an aqueous phase containing a surfactant (emulsifying agent) to stabilize the dispersion.

In this study, SLN-CSEO-APs were prepared using the hot homogenization method with slight modifications, as summarized in Table 1. The lipid phase was obtained by melting stearic acid (2% w/w) at 70°C, followed by the incorporation of CSEO (0.3% w/w) and ethanol (10% w/w). The aqueous phase was prepared by dissolving Tween 20 (5% w/w) and PEG 400 (5% w/w) in 50 mL of distilled water, which was preheated to a temperature near the lipid melting point. The aqueous phase was combined with the lipid phase and reheated to approximately 70°C. Subsequently, 100 mL of distilled water was added, and the mixture was homogenized using a T 25 digital ULTRA-TURRAX® disperser (IKA, Germany) at 25,000 rpm for 5 minutes to form a primary emulsion. The obtained emulsion was dispersed into cold distilled water (4°C) containing 8% lactose in a 1:1 ratio, followed by homogenization at 10,000 rpm for 5 minutes using the same disperser. The final dispersion was stored at −20°C and subjected to a freeze-drying process to obtain dry SLN.[16-17]

Preparation of SLN-CSEO-APs Serum Hydrogel

Serum hydrogel preparation was carried out by combining active ingredients with gelling agents to achieve a soft and flexible consistency, particularly suitable for cosmetic applications, as shown in Table 2.

The formulation of SLN-CSEO-APs serum hydrogel started with the dispersion of Carbopol 940 into distilled water, allowing sufficient time for complete hydration and polymer swelling. In parallel, methylparaben and propylparaben were dissolved in propylene glycol under gentle heating to ensure full solubilization and the formation of a homogeneous preservative solution. The two prepared mixtures, namely the hydrated Carbopol dispersion and the paraben–propylene glycol solution, were combined and thoroughly mixed using an RW 20 digital overhead stirrer (IKA, Germany) at 150 rpm for 10 minutes to obtain a uniform hydrogel base. Following homogenization, SLN-CSEO-APs were incorporated into the hydrogel matrix and mixed until complete uniformity was achieved. The resulting preparation was a stable SLN-CSEO-APs serum hydrogel, which was subjected to further evaluation.[18]

Evaluation of Physical Characteristics of SLN-CSEO-APs

Malvern Panalytical Particle Size Analyzer (PSA, Zetasizer Pro) was used to determine particle size, polydispersity index (PDI), and zeta potential of the prepared formulations. For analysis, the samples were diluted with distilled water at a ratio of 1:25 and mixed using a vortex mixer for 2-3 minutes to ensure uniform dispersion. Subsequently, 2.5 mL of the diluted sample was transferred into a cuvette, which was positioned in PSA holder for particle size and PDI measurements. For zeta potential determination, the sample was loaded into a specialized cuvette designed for electrophoretic mobility analysis, which was kept in PSA holder for measurement.[20-21] In addition, the morphology of SLN-CSEO-APs was examined using a JEOL JEM-1010 Transmission Electron Microscope (TEM). Approximately 500 µL of the sample suspension was carefully dropped onto a copper grid and allowed to adhere. Excess fluid was removed by blotting with vacuum-assisted filter paper, then the grid was subjected to TEM observation to visualize nanoparticle morphology.[11,22-23]

Evaluation of Organoleptic Characteristics of SLN-CSEO-APs

Organoleptic evaluation was carried out to assess the sensory attributes of the serum hydrogel, including the color, shape, and odor. The assessment was performed by placing the sample in a 100 mL beaker, followed by visual observation to evaluate the color and shape, and olfactory examination to determine the specific odor.[19]

Evaluation of Physical Characteristics of SLN-CSEO-APs Serum Hydrogel

Homogeneity test was performed by evenly spreading 1 mg of the sample onto a glass slide, covering it with another slide, and examining the preparation under visual observation to detect the presence of coarse particles.[24] The pH measurement was conducted using a digital pH meter (Horiba Laqua) previously calibrated with standard buffer solutions at pH 4 (acidic), pH 7 (neutral), and pH 10 (basic). The electrode was immersed in the sample, and the measurement was recorded when a stable reading was obtained.[25] The viscosity of the preparation was determined using a Brookfield DV-1 Prime Viscometer equipped with an S63 spindle. The spindle was immersed in 100 mL of the sample kept in a glass beaker and operated at a 30 rpm rotational speed.[22] The spreadability test was performed by positioning 0.5 g of the preparation between two glass plates. A weight ranging from 0 to 2 kg was applied for 1 minute, after which the spread formulation diameter was measured.[26] The adhesion test was conducted by placing 0.5 g of the preparation between two glass slides and applying a load of 500 g for 5 minutes. Following the weight removal, the time required for the slides to detach under an additional 80 g load was recorded as the measure of adhesion.[27]

Stability Test of SLN-CSEO-APs

The stability of SLN-CSEO-APs was evaluated by storing the preparation in airtight trilaminate bags at refrigerated conditions (2-8°C) for 1 month. The use of trilaminate bags was intended to minimize exposure to environmental factors that could compromise the stability of the preparation. The sealed samples were placed in a temperature-controlled refrigerator, and evaluations were performed at weekly intervals all through the storage period. At each interval, samples were examined to monitor potential changes in both physical and chemical characteristics. The assessed parameters included

particle size, PDI, and zeta potential, which were measured using Malvern Panalytical PSA and Zetasizer Pro.[11]

Antioxidant Testing of SLN-CSEO-APs Serum Hydrogel Preparation Using DPPH Method

A total of 40 µL of SLN-CSEO-APs preparation at different concentrations of 100, 50, 25, 12.5, 6.25, and 3.13 ppm was mixed with 40 µL of a 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution formulated in methanol. The mixtures were transferred into a 96-well plate and thoroughly mixed to ensure homogeneity, then incubated at room temperature (approximately 25°C) for 30 minutes. During incubation, the characteristic purple color of DPPH solution gradually diminished as antioxidant compounds in SLN-CSEO-APs preparation scavenged DPPH free radicals. Subsequently, the absorbance of each mixture was measured at 517 nm using a Shimadzu UV-1900 UV-VIS spectrophotometer. Antioxidant activity of SLN-CSEO-APs was expressed as ascorbic acid equivalent (mg AAE/g dry weight), calculated against a standard calibration curve of ascorbic acid. For comparison, antioxidant activity of ascorbic acid was evaluated at lower concentrations of 6, 5, 4, 3, 2, and 1 ppm. These data were used to determine and compare antioxidant capacity of SLN-CSEO-APs preparations across the tested concentrations.[28]

Antioxidant Testing of SLN-CSEO-APs Serum Hydrogel Preparation Using ABTS Method

A total of 5 mL of ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) solution at a concentration of 7 mmol/L was mixed with 88 µL of potassium persulfate solution (140 mM) to generate $ABTS^+$ free radicals (ABTS cation radicals) for use in the conduction of antioxidant activity assay. The mixture was kept in the dark at room temperature for 16 hours to allow complete formation of $ABTS^+$ radicals. The resulting $ABTS^+$ solution was diluted with ethanol until the absorbance reached 0.7 at 734 nm. For antioxidant activity measurement, 10 µL of SLN-CSEO-APs preparation was mixed with 290 µL of the diluted $ABTS^+$ solution in a 96-well plate. The mixture was incubated under dark conditions at room temperature for 6 minutes to achieve a stable reaction. Following incubation, the absorbance was measured at 734 nm using a Shimadzu UV-1900 UV-VIS spectrophotometer. The value of antioxidant activity of SLN-CSEO-APs was calculated relative to a Trolox standard and expressed as milligrams of Trolox equivalent per gram of dry weight sample (mg TE/g dw). A standard calibration curve was constructed using serial concentrations of SLN-CSEO-APs (1000, 500, 250, 125, 62.5, and 31.25 ppm) and compared against Trolox standards at lower concentrations (250, 125, 62.5, 31.25, 15.63, and 7.81 ppm).[29]

Preparation of SLN-CSEO-APs Serum Hydrogel for UVB and UVA In-vitro Testing

The *in-vitro* evaluation of UV protection effectiveness was performed using a UV-Vis spectrophotometer. Approximately 0.05 g of each sunblock and sunscreen cream was accurately weighed and diluted using 96% ethanol, then mixed with chloroform at ethanol-to-chloroform ratios of 1:1 and 1:4, respectively, to obtain a final volume of 10 mL, which corresponded to a concentration of 5000 ppm. The prepared solutions were transferred into quartz cuvettes, and the absorbance was measured across the wavelength range of 290–400 nm at 5 nm intervals using a UV-Vis spectrophotometer. The absorbance values obtained were recorded and used to calculate Sun Protection Factor (SPF, UVB region) based on Mansur formula in Equation 1. [30] Similarly, Protection Grade of UVA (PA) was determined using the calculation method presented in Equation 2.

$$SPF(UVB) = CF \times \sum_{290}^{320} EE\,(\lambda) \times I(\lambda) \times abs\,(\lambda) \quad \ldots\ldots (1)$$

Where CF denotes the correction factor, which has a value of 10. EE (λ) represents the erythemogenic effect of radiation at a given wavelength (λ), while abs (λ) refers to UV spectrophotometric absorbance measured at the corresponding wavelength.

$$PA(UVA) = \sum_{320}^{400} P(\lambda) \times I(\lambda) \times 10^{-A} \quad \ldots\ldots (2)$$

P (λ) denotes the effectiveness of sunscreen in protecting the skin from UVA radiation at a given wavelength (λ). I (λ) represents the intensity of UV radiation at wavelength λ, while 10^{-A} serves as the correction factor signifying the proportion of UVA radiation absorbed by sunscreen. The calculation of Protection Grade of UVA reflects the ability of sunscreen to protect against UVA radiation in the wavelength range of 320-400 nm. The absorbance of the product in this region is quantified by raising the absorbance value to 10^{-A} and multiplying by the corresponding radiation intensity across the specified wavelength range.

Results and Discussion

Organoleptic Characteristics of SLN-CSEO-APs

The organoleptic evaluation of SLN-CSEO-APs preparation is presented in Figure 2A. Prior to the freeze-drying process, the preparation had a milky white appearance with a characteristic odor of CSEO. The dispersion showed slight viscosity and density, with no evidence of phase separation or color heterogeneity after 24 hours of storage. Following freeze-drying, the preparation was converted into a milky white powder that retained the distinctive odor of CSEO and had a slightly fatty texture, as shown in Figure 2B.

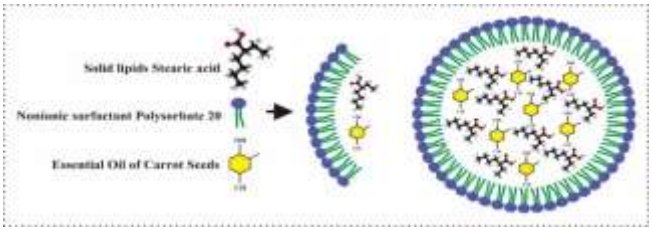

Figure 1: Modified carrot seed essential oil delivery system SLN-CSEO-APs preparation

Table 1: Formulation of SLN-CSEO-Aps

Mixture	Material	Concentration (w/v)
	CSEO	0.3 %
Lipid phase	Stearic acid	2 %
	Ethanol 70%	10 %
	PEG 400	5 %
Aqueous phase	Tween 20	5 %
	Aquades	50 mL

Table 2: Formulation of serum hydrogel SLN-CSEO-APs

Material	Concentration (w/v)	Function
SLN-CSEO-APs	10 %	Active substance
Carbopol 940	0.3 %	Gelling agent
Methyl paraben	0.2 %	Preservative
Propyl paraben	0.4 %	Preservative
Propylene glycol	13 %	Humectant
Triethanolamine	q.s	Alkalizing agent
Aquadest	add 100 mL	Solvent

Physical Characteristics of SLN-CSEO-APs

Particle size analysis of SLN-CSEO-APs serum showed that nanoparticles were in the ideal size range of 100-300 nm, considered optimal for SLN (Table 3). Although SLN particle sizes can generally vary between 50 and 1000 nm, the most effective and widely applied range is often between 100-300 nm. Particle size plays a critical role in determining the physicochemical properties, stability, and functional performance of nanoparticles, particularly the ability to penetrate biological tissues and interact with target cells.[31-32] SLN-CSEO-APs in this study were prepared using the hot homogenization method, in which the lipid phase was heated above the melting point and combined with CSEO. Hot homogenization promotes efficient particle disruption and facilitates the incorporation of active ingredients. The method is

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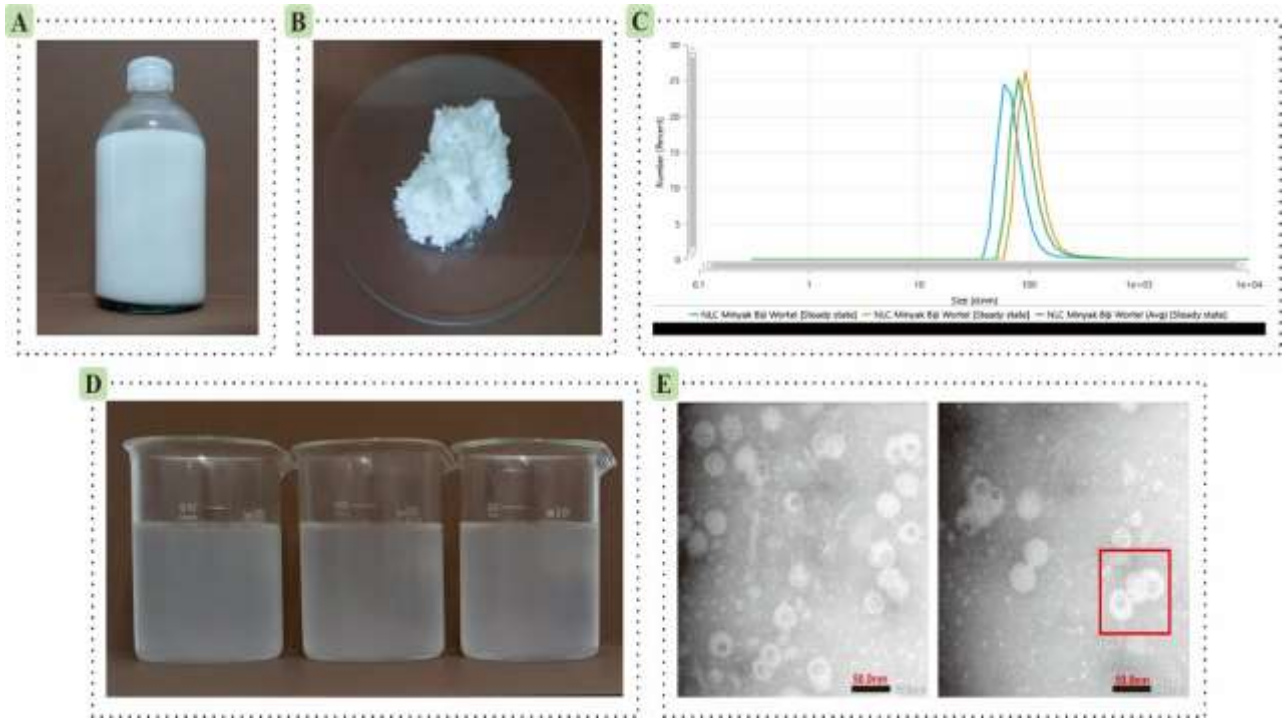

Figure 2: The evaluation results of the physical characteristics of SLN-CSEO-APs preparation are as follows: A) SLN-CSEO-APs sebelum proses freeze-drying untuk menstabilkan sediaan, B) SLN-CSEO-APs powder after freeze-drying treatment, C) Particle size distribution from three observations, D) Hydrogel-based SLN-CSEO-APs serum (one preparation divided into three samples), and E) TEM morphology image of SLN-CSEO-APs.

conducted under controlled heating conditions that consider both the melting point of the lipids and the thermal stability of the active compounds.[32-33] Furthermore, the formation of nanoparticles follows a bottom-up method, where surfactant molecules assemble into SLN matrix, followed by homogenization. This structural organization allows SLN to act as a protective matrix and an effective carrier system for active compounds, such as CSEO, enhancing the stability, bioavailability, and total therapeutic potential.[34]

Figure 2C presents the results of repeated testing, and the particle deformation observed during peak formation suggests that particle size distribution of SLN-CSEO-APs is relatively heterogeneous. This shows that the preparation contains particles spanning a broad size range, yet distributed adequately in the sample. The observation is supported by the obtained PDI value of 0.501 PDI ± 0.00, signifying that, although variations in particle size are present, the distribution remains within acceptable tolerance limits. PDI in this range reflects a moderate degree of particle size distribution, which is considered suitable for topical applications.[11,35] Furthermore, PDI is an important parameter used to evaluate the extent of size heterogeneity in nanoparticle suspensions or dispersions, and the scale ranges from 0 to 1, with a value of 0 representing a uniform particle size distribution (monodisperse), while values near 1 denote a broad and heterogeneous size distribution (polydisperse). PDI value obtained in this study suggests that SLN-CSEO-APs formulation possesses a balanced distribution profile, ensuring both stability and functional suitability for dermal delivery systems.[11]

The zeta potential value of SLN-CSEO-APs was determined to be -23.19 mV ± 0.81, suggesting that nanoparticles possess a negative surface charge and have moderate colloidal stability. Zeta potential serves as a key indicator of colloidal stability in suspension. Generally, zeta potential values greater than +30 mV or smaller than -30 mV reflect a sufficiently high surface charge, which enhances electrostatic repulsion between particles and prevents aggregation.[11] In this study, the obtained value of -23.19 mV suggests that SLN-CSEO-APs preparation has moderate stability. This level of stability is sufficient to prevent aggregation in the short term, but potential instability tends to occur during extended storage. Zeta potential value is considered acceptable for applications in drug delivery and cosmetics, and higher stability may be required under specific conditions or for long-term storage.[36] Moreover, the negative surface charge of SLN-CSEO-APs can influence the interaction with biological membranes, which are often negatively charged. This electrostatic repulsion affects the ability of nanoparticles to adhere to or penetrate cellular membranes, influencing the bioavailability and targeting efficiency. Understanding this interaction is critical for the rational design of SLN intended for pharmaceutical or biomedical applications.[37]

TEM analysis was conducted to evaluate the morphology and distribution of SLN-CSEO-APs. As presented in Figure 2E, TEM images showed that nanoparticles had a predominantly spherical morphology with a single lipid layer and no evidence of aggregation, consistent with the expected characteristics of lipid nanoparticle formulations.[38] The observed morphology further shows that nanoparticles maintain structural integrity without fragmentation, suggesting the presence of a stable lipid matrix. The range of SLN particle size was observed between 50 and 200 nm, corresponding with the desirable size range for SLN-based applications. Nanoparticles in this range are sufficiently small to facilitate efficient drug delivery and topical application, enhancing bioavailability and therapeutic efficacy. There were no agglomeration or particle fusion signs detected, confirming the successful formulation and physical stability of SLN. In TEM micrographs, the lighter (white) regions represent areas with greater electron transparency, typically corresponding to lower-density zones such as interparticle spaces or less compact portions of the lipid matrix. The darker regions correspond to denser structures, specifically SLN, which scatters more electrons and therefore appears darker. These observations confirm that SLN maintains a compact and stable structure in the lipid matrix, ensuring the suitability as a delivery system for bioactive compounds [11,38]

Physical Characteristics of SLN-CSEO-APs Serum Hydrogel

The organoleptic evaluation of SLN-CSEO-APs serum hydrogel showed a cloudy white appearance with a slightly viscous consistency.

There was no phase separation or discoloration observed after 24 hours of storage, as presented in Figure 2D. Homogeneity testing further confirmed a uniform structure, with no evidence of aggregation or uneven distribution of the hydrogel components across the entire preparation.

SLN-CSEO-APs serum hydrogel pH was measured at 5.1 ± 0.5, which fell in the physiologically acceptable range of 4.5-6.5 for facial skin.[39] A pH in this range is considered suitable for topical applications because of the lower potential to cause irritation and the ability to minimize the risk of discomfort during use. Therefore, SLN-CSEO-APs serum hydrogel preparation can be regarded as dermatologically compatible and safe for facial application.[39]

The viscosity of SLN-CSEO-APs serum hydrogel was measured at 4453.7 cP ± 504.9, which fell in the acceptable range for serum hydrogel-based preparations between 2000 and 50,000 cP.[40] The viscosity of the preparations is closely influenced by the concentration of the gelling agent (Carbopol 940) and other excipients, such as triethanolamine. In general, higher concentrations of these components lead to increased viscosity of the final preparation.[41]

According to the test results in Table 4, SLN-CSEO-APs serum hydrogel had a spreadability range of 4-11 cm. A higher spreadability value reflects an improved ability of the preparation to distribute across the skin surface, enhancing contact between the active compound and the skin. This characteristic is important, as spreadability influences both the absorption and the release profile of the active substances, affecting the efficacy of the preparation and user comfort.[42]

Table 3: Results of particle size, polydispersity index and zeta potential testing

	Particle Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
Test Results[a]	257.12 ± 28.82	0.501 ± 0.00	-23.19 ± 0.81

Table 4: Results of spreadability testing

Addition of Load	Spreadability (cm)	Weight of Cover Glass (gram)
0 gr	4.5	
50 gr	5.2	
100 gr	6.7	
150 gr	7.3	
200 gr	8.4	150.0
500 gr	9.8	
1 kg	10.3	
2 kg	11.8	

The adhesion test produced a value of 1.1 s ± 0.1, which met the requirement of being greater than 1 second. This result shows that SLN-CSEO-APs serum hydrogel has adequate adhesion to the skin. Adhesion is known to be positively correlated with viscosity, as higher viscosity tends to increase adhesion strength. However, excessive adhesion may block skin pores, and insufficient adhesion can reduce the bioavailability and total effectiveness of the active compound.[43] These results suggest that SLN-CSEO-APs serum hydrogel preparation shows an optimal balance between viscosity, spreadability, and adhesion, supporting the suitability for topical application.

SLN-CSEO-APs Serum Hydrogel Stability Test

Particle size results from the stability test showed no significant difference compared to the control during the first week. However, significant changes were observed in subsequent weeks, with p-values of <0.05 and <0.01 in the second and third weeks, respectively, and a highly significant difference in the fourth week ($p < 0.0001$). As presented in Figure 3A, these results show that particle size remained in the optimal range for dermal penetration or follicular targeting (100-300 nm) during the first and second weeks. Particle size values exceeded 300 nm in the third and fourth weeks,[31] suggesting that prolonged storage induced significant changes in particle characteristics. The observed increase in particle size during storage may be attributed to several factors, even under low-temperature conditions of 2-8°C. A contributing factor is particle coalescence, in which smaller nanoparticles gradually merge over time due to thermal motion, leading to the formation of larger particles. Additionally, lipid redistribution or migration in nanoparticle matrix can alter both particle size and size distribution. The changes are influenced by the intrinsic physicochemical properties of the lipids used in the preparation. Refrigeration slows the kinetics of emulsion instability but cannot completely prevent instability. These mechanisms collectively contribute to particle size enlargement during extended storage.[44]

PDI measurements during the stability test showed no significant difference compared to the control in the first week. Substantial differences were observed in the second and third weeks, with significance levels of $p < 0.05$ and $p < 0.01$, respectively. By the fourth week, a highly significant difference was observed ($p < 0.001$). Despite PDI being in the generally acceptable range, values exceeding 0.5-0.7 are considered suboptimal due to reflecting a broader particle size distribution and potential instability in the preparation.[11,22] As presented in Figure 3B, PDI values increased consistently from week one through week four. This progressive rise can be attributed to particle aggregation or clustering during storage, where smaller nanoparticles combine to form larger particles, leading to greater variability in size distribution. Another contributing factor may be the process of Ostwald ripening, in which smaller particles gradually dissolve and the material is redeposited onto larger particles. This phenomenon causes smaller particles to diminish or disappear, while larger particles continue to grow, broadening the size distribution and elevating PDI value.[32,45]

Zeta potential values obtained from the stability test showed no significant difference compared to the control during the first week. However, substantial reductions were observed in the second and third weeks ($p < 0.05$), which became more pronounced in the fourth week ($p < 0.01$), as presented in Figure 3C. These changes may be attributed to the adsorption of molecules from the storage medium or impurities onto nanoparticle surface, capable of modifying the surface charge and reducing the zeta potential. In addition, degradation of lipid components or other excipients, such as surfactants, may contribute to the decline in surface charge, lowering zeta potential of the preparation. The observed trend suggests a correlation between particle size, PDI, and zeta potential values. The aggregation and coalescence of smaller particles into larger structures reduces the total surface area available, thereby decreasing the surface charge density. This reduction in surface charge weakens electrostatic repulsion among particles, leading to a decline in zeta potential and an increased risk of instability during extended storage.[36,44-45]

SLN-CSEO-APs Serum Hydrogel Antioxidant Test

DPPH assay evaluates antioxidant activity based on the interaction between hydrogen atoms donated by antioxidant compounds and the unpaired electrons of the radical compound diphenylpicrylhydrazyl (DPPH). This reaction converts DPPH into the non-radical form, diphenylpicrylhydrazine,[46] and the reduction process is visually shown by a color change in the solution from purple to yellow, which reflects the neutralization of free radicals by antioxidant.[47] The occurrence of antioxidant activity in DPPH assay is commonly expressed in the form of the IC_{50} value, defined as the concentration of the sample required to scavenge 50% of DPPH radicals.[48] IC_{50} value of SLN-CSEO-APs serum was 43.540 ppm, and IC_{50} value of ascorbic acid, used as a reference standard, was 6.829 ppm (Table 5). A lower IC_{50} value corresponds to stronger antioxidant activity.[49] These results show that SLN-CSEO-APs serum possesses substantial antioxidant potential, although the activity is lower compared to pure ascorbic acid. The incorporation of CSEO in SLN system provides enhanced stability and controlled release, making the formulation a promising candidate for applications requiring sustained antioxidant protection in pharmaceutical and cosmetic

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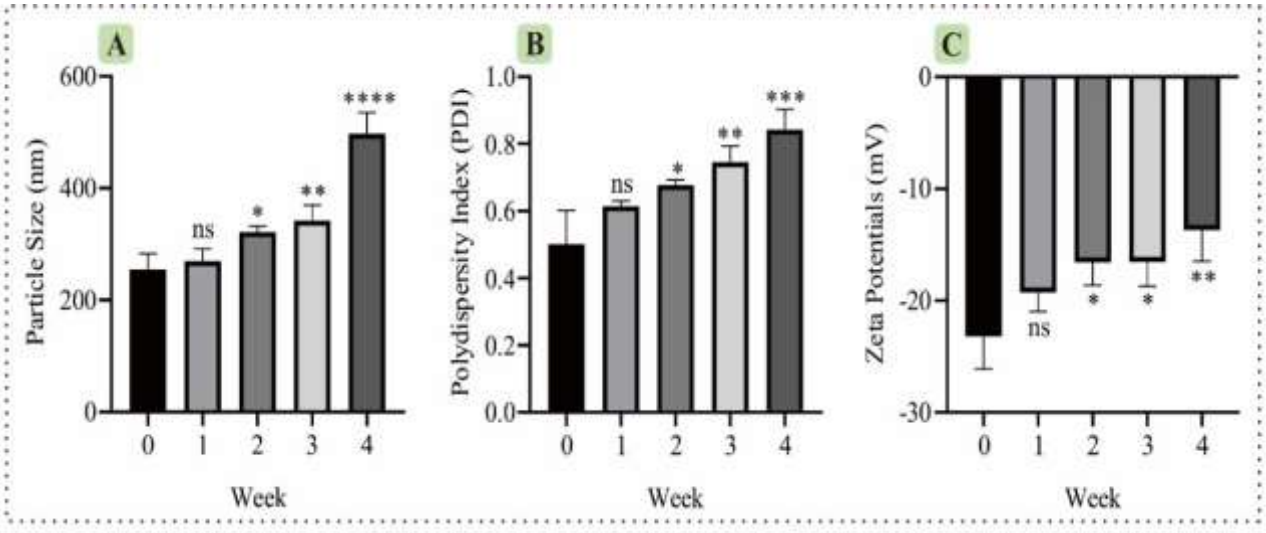

Figure 3: The stability test observation graph includes (A) particle size, (B) polydispersity index, and (C) zeta potential. The samples were analyzed using One-Way ANOVA with a 95% confidence level, followed by a post-hoc test to identify significant differences within each group. Each group was compared to the sample at Week 0. n: 5; ns: not important, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Table 5: Results of SLN-CSEO-APs serum hydrogel Antioxidant testing using DPPH method

No	Sample	LN Concentration (ppm)	% Inhibition[a]	IC_{50} (ppm)
1	Ascorbic acid	4.605	92.454 ± 1.383	6.829
		3.912	80.622 ± 2.572	
		3.219	68.454 ± 1.970	
		2.526	56.924 ± 1.385	
		1.833	47.647 ± 1.261	
		1.139	40.723 ± 2.067	
2	SLN-CSEO-APs serum hydrogel	4.605	57.664 ± 24.887	43.540
		3.912	51.899 ± 20.932	
		3.219	48.218 ± 4.105	
		2.526	32.739 ± 6.988	
		1.833	24.958 ± 2.688	
		1.139	10.655 ± 29.512	

preparations. ABTS assay evaluates antioxidant activity based on the ability of antioxidant to quench ABTS cation radicals, which is reflected by a change in color intensity.[50] ABTS cation radical, a nitrogen-centered species, has a characteristic blue-green color.[51] During the reduction by antioxidant compounds, these radicals are converted into the non-radical form, leading to a gradual loss of color and resulting in a colorless solution. Since the method is light-sensitive, the formation of ABTS cation radicals requires an incubation period of 12-16 hours in the dark to ensure optimal stability and accuracy.[51,52] As presented in Table 6, SLN-CSEO-APs serum showed IC_{50} value of 24.644 ppm, while Trolox standard had IC_{50} value of 19.912 ppm. IC_{50} value below 50 ppm is generally considered to represent strong antioxidant activity. These results suggest that SLN-CSEO-APs serum possesses potent antioxidant capacity, with effectiveness comparable to Trolox standard, thereby supporting the potential application in pharmaceutical and cosmetic preparations requiring antioxidant protection.

The antioxidant activity of SLN-CSEO-APs hydrogel serum was assessed using both DPPH and ABTS methods, showing measurable antioxidant potential. The DPPH assay revealed an IC_{50} of 6.829 ppm for Ascorbic Acid, while the IC_{50} for SLN-CSEO-APs was 43.540 ppm. In this context, 1 mg AAE/g dw is defined as the activity equivalent to 1 ppm of ascorbic acid. Based on this, the mg AAE/g dw value for SLN-CSEO-APs was calculated to be 0.157 mg AAE/g dw. Similarly, the ABTS assay showed an IC_{50} of 19.912 ppm for Trolox, while SLN-CSEO-APs had an IC_{50} of 30.905 ppm. Here, 1 mg TE/g dw is defined as the activity equivalent to 1 ppm of Trolox, and the calculated value for SLN-CSEO-APs was 0.644 mg TE/g dw. These values were determined by comparing SLN-CSEO-APs with standard reference compounds, ascorbic acid for the DPPH assay and Trolox for the ABTS assay, both of which are widely used as benchmarks for evaluating antioxidant activity. The results are consistent with the antioxidant activity classification adapted from Gulcin (2025), [53] the results fall in the moderate activity category, defined by values ranging from 0.5 to 1.0 mg/g dw. In this classification system, values below 0.5 mg/g dw are considered weak, 1.0 to 2.0 mg/g dw are categorized as strong, and >2.0 mg/g dw are classified as very strong. The results showed that SLN-CSEO-APs hydrogel serum has effective antioxidant activity, sufficient to protect against free radicals. Although antioxidant capacity is lower compared to pure standard compounds such as ascorbic acid and Trolox, the formulation still offers significant antioxidant benefits. The incorporation of CSEO into SLN hydrogel system may enhance

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Table 6: Results of SLN-CSEO-APs serum hydrogel Antioxidant testing using ABTS method

No	Sample	LN Concentration (ppm)	% Inhibition[a]	IC_{50} (ppm)
1	Trolox	6.908	98.034 ± 1.096	19.912
		6.215	87.891 ± 1.926	
		5.521	79.125 ± 0.520	
		4.828	70.318 ± 0.828	
		4.135	62.823 ± 0.863	
		3.442	57.266 ± 0.914	
2	SLN-CSEO-APs serum hydrogel	6.908	97.104 ± 1.412	30.905
		6.215	87.040 ± 1.146	
		5.521	75.102 ± 3.825	
		4.828	65.824 ± 1.579	
		4.135	57.686 ± 2.596	
		3.442	53.427 ± 1.558	

[a]Average result of 3 replications ± Standard Deviation

Table 7: Results of UVB and UVA testing *in-vitro* assay

Sample Name	Value			Average	RSD	Category
	1	2	3			
SLN-CSEO-APs / UVB	12.991	12.964	14.133	13.549	0.827	Medium Protection
SLN-CSEO-APs / UVA	4.178	4.180	4.249	4.202	0.040	PA++

stability and prolong antioxidant effects, supporting the potential application in cosmetic and pharmaceutical formulations.

CSEO is well recognized for the strong antioxidant activity, which can be evaluated through assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) methods.[54] Antioxidant potential of CSEO is primarily attributed to the rich chemical composition, which contains a variety of bioactive compounds. CSEO is abundant in carotenoids, particularly β-carotene, a highly potent antioxidant. Carotenoids exert antioxidant effects through the donation of electrons to neutralize free radicals, preventing oxidative damage to cells and biomolecules.[55] In addition, CSEO contains phenolic compounds such as chlorogenic acid, caffeic acid, and ferulic acid, all of which show significant antioxidant activity due to the ability to donate protons and effectively scavenge free radicals.[56] Vitamin E (tocopherol) is another important constituent of CSEO that acts as a lipid-soluble antioxidant to protect cell membranes from lipid peroxidation by capturing peroxyl radicals formed during oxidative processes.[55] Furthermore, CSEO is enriched with sesquiterpenes and monoterpenes, including β-bisabolene and carotol, which contribute to antioxidant activity through mechanisms such as free radical scavenging and inhibition of lipid oxidation.[57] Essential fatty acids, such as linoleic acid and oleic acid, play a role by interacting with and stabilizing free radicals, enhancing the overall antioxidant potential of CSEO.[58] These bioactive constituents show the multifaceted antioxidant mechanisms of CSEO, supporting the potential application in pharmaceutical, nutraceutical, and cosmetic formulations aimed at mitigating oxidative stress and protecting biological systems from free radical-induced damage.

SLN-CSEO-APs Serum Hydrogel for UVB and UVA In-vitro Testing

UV protection testing results for SLN-CSEO-APs preparation showed UVB protection value of 13.5485 ± 0.8273 and UVA protection value of 4.2021 ± 0.0400. UVB value suggests that the preparation is capable of protecting the skin from UVB radiation approximately 13.5 times longer than unprotected skin, leading to the classification in the *moderate protection* category as presented in **Table 7**. UVB protection is a quantitative measure of sunscreen effectiveness, as adequate absorbance in the wavelength range of 290–400 nm is required to prevent sunburn and minimize skin damage.[59] UVA protection value of 4.2021 ± 0.0400 corresponds to PA++ classification, suggesting that the formulation provides effective defense against UVA penetration. This level of protection is essential for reducing the risk of premature skin aging and other UVA-induced damage. PA value was calculated using a constant (C = 1.1), representing the midpoint of the 0.6-1.6 range specified by ISO 24443:2021, due to the absence of in vivo validation studies. In this context, PA++ classification reflects moderate protection against UVA rays. PA system is widely used to quantify the degree of UVA protection provided by sunscreen and skincare products. Each additional "+" symbol represents a higher level of protection, with PA++++ denoting the strongest category. Therefore, PA++ designation confirms that SLN-CSEO-APs preparation offers sufficient UVA defense, complementing UVB protection. This dual functionality shows the potential as a skincare product designed to safeguard the skin from short-term damage in the form of sunburn and long-term effects, including photoaging, DNA alterations, and increased risk of skin cancer.

The incorporation of SLN as a delivery system for the active constituents of CSEO enhances the stability of the bioactive compounds and enables controlled release, improving the total effectiveness of UVB and UVA protection. CSEO contains several phytochemicals with photoprotective properties, including β-carotene, lutein, and zeaxanthin, which act as natural pigments capable of absorbing UV radiation and simultaneously functioning as antioxidant to mitigate UV-induced oxidative damage.[60] In addition, CSEO is rich in vitamin E, a potent lipid-soluble antioxidant that plays a crucial role in protecting skin cells from free radicals generated by UV exposure.[58] The presence of essential fatty acids, such as linoleic acid and oleic acid, further contributes to maintaining skin hydration and reinforcing the natural barrier function of the skin. A well-hydrated and intact skin barrier is better equipped to resist the deleterious effects of UV radiation. CSEO contains phytoestrogens and flavonoids, which promote skin cell regeneration and aid in the repair of UV-induced damage. Flavonoids have both antioxidant and anti-inflammatory properties, providing an additional protective mechanism against UV radiation and free radical-mediated skin injury.[61] Moreover, the folic

acid content in CSEO has been reported to attenuate inflammation and protect the skin from oxidative stress.[58] These bioactive components collectively show the synergistic role of CSEO in enhancing photoprotection when incorporated into SLN. The dual action of direct UV absorption and antioxidant-mediated defense, as shown by SLN-CSEO-APs preparations, signify the potential in preventing sunburn and reducing the risk of long-term UV-related skin damage, including photoaging and carcinogenesis.

Conclusion

In conclusion, SLN preparation containing CSEO as a potential anti-aging and sun protection agent was successfully developed using the hot homogenization method, which produced nanoparticles with stable physical characteristics suitable for topical application. The produced particles had a spherical monolayer morphology without agglomeration, moderate colloidal stability, and an average particle size of 257.12 nm ± 28.82, signifying good potential for dermal penetration. The preparation showed strong antioxidant activity, appearing slightly lower than the activity of the positive control. In terms of UV protection, UVB protection was categorized as moderate, and UVA protection reached PA++ rating, reflecting a satisfactory protective performance. However, the stability assessment showed a gradual decline across all storage parameters over time, suggesting the need for further optimization to improve the long-term stability of SLN-CSEO-APs system.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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