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The Influence of Zinc Oxide Concentration and Stirring Time on the SPF and Physicochemical Properties of Green Tea Leaf Extract Nanoemulgel

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Abstract

This study evaluated the effects of zinc oxide (ZnO) concentration and stirring time on the photoprotective and physicochemical properties of a green tea (*Camellia sinensis*) leaf extract nanoemulgel. Formulations containing 1–5% ZnO were prepared using a self-nanoemulsifying drug delivery system (SNEDDS) with stirring times of 15 and 45 min. The formulations were evaluated for sun protection factor (SPF), pH, density, and homogeneity, followed by two-way analysis of variance. The selected formulation was further characterized for viscosity and particle size. ZnO

concentration and stirring time significantly affected SPF ($p < 0.05$), while ZnO concentration significantly affected pH and density. The 5% ZnO formulation stirred for 15 min exhibited the highest SPF (17.47) and a mean particle size of 147.77 nm. However, phase separation occurred during storage, whereas formulations containing 1–2% ZnO showed better physical homogeneity. Further optimization is therefore required to improve physical stability while maintaining photoprotective performance.

Keywords: *Camellia Sinensis*, Nanoemulgel, Physical Characterization, Sun Protection Factor, Zinc Oxide

Introduction

The skin acts as the primary barrier against environmental factors, including ultraviolet (UV) radiation. Excessive UV exposure can cause erythema, hyperpigmentation, photoaging, and DNA damage, highlighting the importance of sunscreen for photoprotection [1, 2]. The *Sun Protection Factor* (SPF) is widely used to evaluate sunscreen efficacy, particularly against UV-B-induced erythema, with *in vitro* SPF determination by UV-Vis spectrophotometry commonly applied in preliminary sunscreen evaluation [3, 4].

Zinc oxide (ZnO) is a widely used inorganic UV filter that provides broad-spectrum UVA and UVB protection through UV absorption, reflection, and scattering, with favorable photostability [5, 6]. Its photoprotective performance depends on concentration, particle size, and the homogeneity of dispersion. Reducing particle size can increase specific surface area and improve ZnO dispersion, potentially enhancing its UV protection [7, 8].

Nanoemulgel combines the advantages of nanoemulsions and gel matrices, providing a promising topical delivery system [9, 10]. In ZnO-containing formulations, stirring conditions can influence particle dispersion, homogeneity, and physical stability. Insufficient or excessive stirring may cause poor dispersion or particle agglomeration, making process optimization essential for formulation stability [9, 11].

Green tea (*Camellia sinensis*) leaf extract is rich in polyphenolic catechins with antioxidant and photoprotective properties, making it a promising natural active ingredient for topical sunscreen formulations [12, 13]. Researchers have incorporated green tea extract into sunscreen formulations and shown that it contributes to SPF and antioxidant performance [14, 15]. Zinc oxide (ZnO), as an inorganic UV filter, can provide additional photoprotective activity when incorporated into sunscreen formulations. However, studies combining green tea extract and ZnO in nanoemulgel systems, particularly those systematically investigating both ZnO concentration and stirring time, remain limited. Therefore, investigating these formulation and processing variables may provide useful information for optimizing the SPF and physicochemical properties of ZnO-containing green tea nanoemulgels.

Accordingly, this study investigated the effects of ZnO concentration (1–5%) and stirring time (15 and 45 min) on the SPF and physicochemical characteristics of green tea (*Camellia sinensis*) leaf extract nanoemulgels containing 5% extract. SPF was determined *in vitro* using the Mansur method with UV-Vis spectrophotometry, while pH, homogeneity, viscosity, and particle

size were evaluated. Statistical analysis was used to assess the effects of ZnO concentration and stirring time and to support formulation optimization for potential topical sunscreen application.

Material and Methods

Study Design

This study employed a laboratory-based quantitative experimental design with a two-factor arrangement to evaluate the effects of zinc oxide (ZnO) concentration and stirring time on the sun protection factor (SPF) and physicochemical characteristics of a green tea (*Camellia sinensis*) leaf extract nanoemulgel. The nanoemulgel base consisted of a virgin coconut oil (VCO)-based nanoemulsion incorporated into a Carbopol 940 gel matrix, formulated with a fixed green tea extract concentration of 5% (w/w). ZnO concentration was varied at five levels (1, 2, 3, 4, and 5% w/w), and stirring time was evaluated at two levels (15 and 45 min), yielding a 5×2 factorial combination of independent variables. The response variables assessed were SPF, pH, density, homogeneity, viscosity, and particle size. Incorporation of ZnO as an inorganic UV filter into semisolid nanoemulgel systems has been reported in previous studies (citation). All experiments were conducted under controlled laboratory conditions at the Chemical Engineering Laboratory, State Polytechnic of Malang (Politeknik Negeri Malang), Indonesia.

Materials and Equipment

Fresh green tea leaves (*Camellia sinensis*) were used as the plant material. The formulation materials included 96% ethanol, ZnO, virgin coconut oil (VCO), Tween 80, polyethylene glycol 400 (PEG 400), Carbopol 940, propylene glycol, triethanolamine (TEA), glycerin, and distilled water, with pharmaceutical- or analytical-grade materials used where applicable.

The main equipment included an analytical balance, 60-mesh sieve, maceration vessel, rotary evaporator, magnetic stirrer, pH meter, viscometer, particle size analyzer (PSA), UV-Vis spectrophotometer, drying oven, mortar and pestle, centrifuge, and standard laboratory glassware.

Preparation and Extraction of Green Tea Leaves

Approximately 500 g of fresh green tea leaves were washed, air-dried, and oven-dried at 40–50 °C to constant weight. The dried leaves were pulverized and sieved through a 60-mesh sieve, and the resulting powder was stored in a dry, closed container. The extraction method was based on previously reported maceration approaches with modifications. For extraction, 100 g of the powder was macerated with 96% ethanol at a 1:10 (w/v) solid-to-solvent ratio for 24 h at room temperature without heating. The mixture was filtered, and the residue was re-extracted with fresh solvent under the same conditions. Maceration was repeated for three 24-h cycles, and the combined filtrates were concentrated under reduced pressure using a rotary evaporator. The resulting green tea leaf extract was stored in a tightly closed container until further formulation.

Preparation of the Nanoemulsion System

The nanoemulsion system was prepared using VCO (3% w/w) as the oil phase, Tween 80 (4% w/w) as the surfactant, and PEG 400 (2% w/w) as the co-surfactant. The components were mixed and homogenized using a magnetic

stirrer at 2000 rpm for 30 min at 25 ± 2 °C. Green tea leaf extract was then incorporated at 5% (w/w). ZnO was prepared at concentrations of 1–5% (w/w) and pre-dispersed to facilitate uniform particle distribution. The ZnO dispersion was gradually incorporated into the extract-containing nanoemulsion under continuous stirring, followed by homogenization for an additional 30 min. The resulting formulation was visually examined for macroscopic appearance, aggregate formation, and phase separation as preliminary indicators of physical uniformity.

Preparation of Green Tea Extract–ZnO Nanoemulgel

Carbopol 940 (1.5% w/w) was dispersed in distilled water and hydrated for 30 min, followed by the addition of propylene glycol (5% w/w). The previously prepared green tea extract–ZnO nanoemulsion was gradually incorporated into the hydrated gel base under continuous stirring. Distilled water was added to obtain a final formulation weight of 100 g, followed by gradual addition of triethanolamine (1% w/w) to neutralize Carbopol 940 and facilitate gel formation. The resulting nanoemulgel was homogenized using a magnetic stirrer at 200 rpm for 15 or 45 min according to the experimental treatment. The formulations were subsequently evaluated for physicochemical properties and *in vitro* sun protection factor (SPF).

Nanoemulgel Formulation

The composition of the nanoemulgel formulations is presented in Table 1.

Table 1: Composition of the ZnO-loaded nanoemulgel formulations

Composition (% w/w)	F1	F2	F3	F4	F5
Green Tea Leaf Extract	5	5	5	5	5
Virgin Coconut Oil	3	3	3	3	3
Tween 80	4	4	4	4	4
PEG 400	2	2	2	2	2
Carbopol 940	1.5	1.5	1.5	1.5	1.5
Propylene Glycol	5	5	5	5	5
Triethanolamine	1	1	1	1	1
Zinc Oxide	1	2	3	4	5
Glycerin	5	5	5	5	5
Purified Water	q.s	q.s	q.s	q.s	q.s

Physicochemical Characterization of Nanoemulgel Homogeneity Assessment

For homogeneity assessment, 0.1-g samples were collected from the upper and lower portions of each formulation, diluted to 10 mL with distilled water, and homogenized. Transmittance was measured in duplicate at 650 nm using a UV-Vis spectrophotometer, with distilled water as the blank. The mean transmittance difference between the two portions was used as an indicator of dispersion uniformity, with smaller differences indicating greater uniformity.

Particle Size Analysis

Particle size and distribution were measured using a BIOBASE BK-802N Particle Size Analyzer employing dynamic light scattering (DLS). Samples were diluted according to the manufacturer's instructions and analyzed in clean, bubble-free cuvettes at controlled temperature using a 532 nm laser and 90° detection angle. The mean hydrodynamic diameter and particle size distribution were

obtained from Brownian diffusion based on the Stokes–Einstein relationship.

pH Measurement

The pH of the nanoemulgels was determined using a calibrated digital pH meter. Approximately 1 g of each sample was dispersed in 10 mL of distilled water and homogenized prior to measurement. The pH meter was calibrated using standard buffer solutions according to the manufacturer's instructions. Measurements were performed in duplicate after a stable reading was obtained, and the mean pH value was reported.

Density Measurement

The density of the nanoemulgels was determined using a calibrated pycnometer. The pycnometer was cleaned, dried, and weighed before being filled with the sample to the calibrated volume, avoiding air bubbles. The filled pycnometer was then weighed, and density was calculated as the ratio of sample mass to pycnometer volume. Measurements were performed in duplicate, and the results were expressed as mean density values (g/mL).

Viscosity Measurement

The viscosity of the nanoemulgel was measured using a Brookfield viscometer with spindle 40Z at 10 rpm. Approximately 60 g of sample was placed in the measurement container, ensuring adequate spindle immersion. The sample was equilibrated under the measurement conditions, and the viscosity was recorded after a stable reading was obtained (approximately 2–3 min). The selected formulation was measured once at an external testing laboratory according to its standard operating procedure. Viscosity was reported in mPa·s.

In Vitro Sun Protection Factor Determination

The *in vitro* sun protection factor (SPF) of the nanoemulgels was determined by UV–Vis spectrophotometry using the Mansur method. Samples were diluted with 96% ethanol to obtain absorbance values within the linear range of the instrument. The nanoemulgel base without ZnO and green tea leaf extract was prepared using the same procedure and used as the matrix blank. Before measurement, samples were gently homogenized to ensure uniform dispersion. Absorbance was measured in duplicate from 290 to 320 nm at 5-nm intervals using a quartz cuvette under identical conditions. The mean absorbance values were used to calculate SPF according to the Mansur equation using the reported erythral effect and solar intensity weighting factors. A correction factor of 10 was applied. Consistent dilution, measurement, and homogenization procedures were used for all formulations to minimize scattering-related variability associated with ZnO.

$$SPF = CF \times \sum_{290}^{320} [EE(\lambda) \times I(\lambda) \times A(\lambda)]$$

Where:

- CF = correction factor, with a value of 10;
- $EE(\lambda)$ = erythral effect spectrum at wavelength λ ;
- $I(\lambda)$ = solar intensity spectrum at wavelength λ ;
- $A(\lambda)$ = measured absorbance at wavelength λ .

Results and Discussion

Preparation and Extraction of Green Tea Leaves

The green tea leaves (*Camellia sinensis*) were successfully processed into a dried powder by washing, oven-drying at 50 °C for 24 h, pulverization, and sieving through a 60-mesh sieve. From 200 g of dried leaf powder, maceration with 96% ethanol yielded 86.2767 g of concentrated extract, corresponding to an extraction yield of 43.14%. The obtained yield indicates substantial recovery of ethanol-soluble constituents under the applied extraction conditions. Green tea contains various bioactive compounds, particularly catechins and other phenolic constituents, and their recovery is strongly influenced by solvent composition and extraction conditions [16, 17]. The use of ethanol is appropriate for recovering phenolic and catechin-rich constituents from *C. sinensis*, as demonstrated in previous extraction studies [18]. However, the extraction yield represents the total mass of concentrated extract and does not directly indicate the concentration or recovery of individual bioactive compounds. The resulting extract was subsequently used as the botanical active ingredient in the nanoemulgel formulation.

Green Tea Leaf Extract Nanoemulgel Formulation and Optimization

Green tea leaf extract (*Camellia sinensis*) nanoemulgels were successfully formulated using a SNEDDS consisting of VCO, Tween 80, and PEG 400 as the oil phase, surfactant, and co-surfactant, respectively. Similar SNEDDS systems using VCO, Tween 80, and PEG 400 have been reported to produce nanoemulsions with favorable physicochemical characteristics and stability [19, 20]. The SNEDDS composition was maintained constant across formulations to isolate the effects of ZnO concentration and stirring time. ZnO was pre-dispersed in glycerin and incorporated into the nanoemulsion before dispersion in a Carbopol 940-based gel. Similar ZnO-loaded SNEDDS–Carbopol nanoemulgel systems have been investigated for topical photoprotection [21]. All formulations initially exhibited acceptable gel consistency.

During storage, ZnO sedimentation and phase separation were observed, particularly at higher ZnO concentrations. This behavior suggests that increased ZnO loading may compromise dispersion stability within the gel matrix. The physical stability of nanoemulgels can be affected by particle size distribution, viscosity, polymer concentration, and interactions among formulation components [21]. Thus, ZnO concentration appears to be an important formulation factor affecting physical stability, whereas stirring time may contribute to dispersion uniformity. Optimization should therefore consider both photoprotective performance and physical stability to obtain a formulation with balanced functional and physicochemical properties.

pH Measurement

The pH values of the green tea leaf extract nanoemulgels ranged from 5.5 to 6.5, indicating that all formulations were within the pH range specified for topical sunscreen preparations (4.5–8.0). Increasing ZnO concentration generally resulted in a slight increase in pH, with formulations containing 3–5% ZnO stirred for 15 min showing relatively stable values around pH 6. This trend may be associated with the amphoteric nature of ZnO. In contrast, increasing the stirring time from 15 to 45 min

produced only minor changes in pH, suggesting that stirring primarily affected physical dispersion rather than the acid–base characteristics of the formulations.

Two-way ANOVA showed that ZnO concentration significantly affected pH ($p = 0.038$), whereas stirring time had no significant effect ($p = 0.070$). These results indicate that pH variation was primarily associated with ZnO concentration. Nevertheless, all formulations remained within the specified topical pH range, suggesting that pH was not a limiting factor under the investigated formulation conditions. The observed trend is consistent with previous studies reporting that formulation composition can influence the pH of ZnO-containing nanoemulgel systems [21, 22].

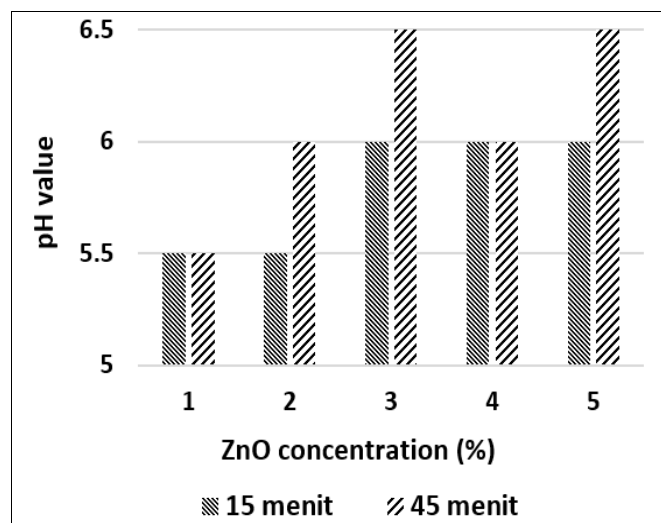


Fig 1: The Influence of Zinc Oxide Concentration and Stirring Time on pH Value

Density

The density of the green tea extract nanoemulgels ranged from 1.025 to 1.052 g/mL and increased with increasing ZnO concentration. This trend can be attributed to the relatively high density of ZnO (5.61 g/cm³), which increases the mass of the formulation at a given volume [23]. The influence of ZnO concentration on density is particularly relevant in sunscreen formulations because increasing mineral UV-filter loading can substantially alter formulation density and, consequently, the effective film thickness during SPF testing [23].

Two-way ANOVA confirmed that both ZnO concentration ($p < 0.001$) and stirring time ($p = 0.001$) significantly affected density, indicating that both formulation composition and processing conditions contributed to the observed variation. According to SNI 16-4399-1996, sunscreen preparations have a specific-gravity specification of 0.95–1.05 g/mL. Most formulations were within this range, whereas the highest density (1.052 g/mL) slightly exceeded the upper limit. Therefore, ZnO loading should be carefully controlled during formulation optimization to balance UV-filter incorporation with the desired physical characteristics of the nanoemulgel.

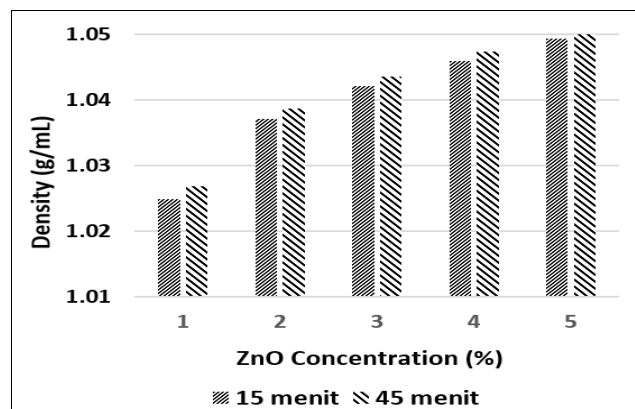


Fig 2: The Influence of Zinc Oxide Concentration and Stirring Time on Density Value

Homogeneity

The nanoemulgels exhibited transmittance differences of 0.40–1.39%, indicating relatively uniform dispersion after redispersion. Neither ZnO concentration ($p = 0.102$) nor stirring time ($p = 0.910$) significantly affected homogeneity. Although 15 min of stirring was sufficient to achieve uniform dispersion, formulations containing 3–5% ZnO showed sedimentation and phase separation during storage, unlike those containing 1–2% ZnO. The observed sedimentation may be associated with changes in particle dispersion and ZnO aggregation, which are strongly influenced by the physicochemical conditions of the dispersion medium.

Previous studies have demonstrated that ZnO nanoparticle aggregation and dispersion stability are sensitive to physicochemical parameters of the surrounding medium, including pH and ionic strength [24]. In sunscreen formulations, increasing metal oxide content may also promote particle aggregation and agglomeration, while the dispersion vehicle substantially affects the resulting colloidal stability [25]. The effect of ZnO concentration on physical stability is therefore relevant to sunscreen formulation optimization. Thus, the spectrophotometric homogeneity results primarily reflected post-redispersion uniformity rather than storage stability. These findings indicate that ZnO concentration should be optimized to achieve adequate dispersion while maintaining physical stability during storage.

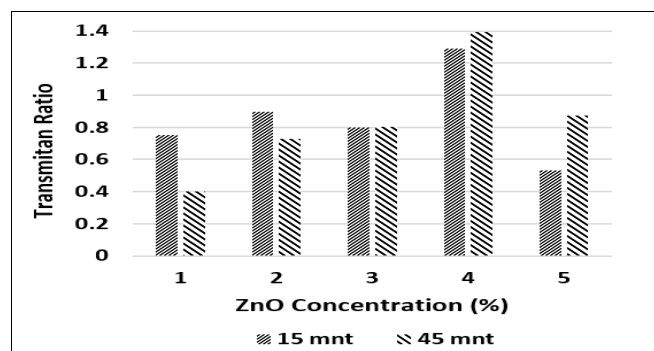


Fig 3: The Influence of Zinc Oxide Concentration and Stirring Time on Homogeneity

Sun Protection Factor

The *in vitro* SPF of the nanoemulgels was significantly affected by ZnO concentration ($p = 0.003$) and stirring time ($p = 0.031$). SPF increased with ZnO concentration, reaching a maximum of 17.48 in the 5% ZnO formulation after 15 min of stirring, whereas prolonged stirring to 45 min was associated with lower SPF values. The enhanced SPF at higher ZnO concentrations may be attributed to increased attenuation of UV radiation by ZnO [26]. While the reduction following prolonged stirring may reflect changes in particle dispersion and possible agglomeration [27]. This interpretation should be considered cautiously because particle aggregation was not directly characterized in the present study.

However, higher ZnO concentrations were accompanied by sedimentation and phase separation during storage. As the samples were re-homogenized before SPF analysis, the measured values represent photoprotective efficacy following redispersion and should not be interpreted as indicators of storage stability. This distinction is particularly relevant because *in vitro* SPF measurements of ZnO-containing formulations can be affected by formulation characteristics and may not directly correspond to *in vivo* SPF values [26]. Overall, the results demonstrate that ZnO concentration and processing conditions should be jointly optimized to achieve effective UV protection while maintaining formulation stability.

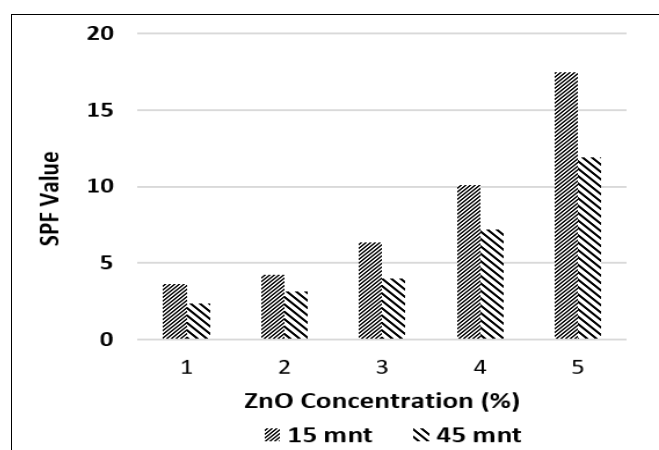


Fig 4: The Influence of Zinc Oxide Concentration and Stirring Time on SPF Value

Formulation Optimization

Response optimization identified 5% ZnO with 15 min of stirring as the statistically optimal formulation, with a composite desirability of 0.5849. The predicted SPF (17.4759) closely matched the experimental value (17.48). However, this formulation exhibited sedimentation and phase separation during storage, whereas 1–2% ZnO formulations showed better physical homogeneity but lower SPF. Thus, the optimized formulation represented the best compromise among the modeled responses but did not ensure adequate storage stability, highlighting the need to incorporate physical stability into subsequent optimization.

Viscosity

The selected nanoemulsion showed a viscosity of 13.2–13.8 mPa·s, decreasing to 6.85–6.92 mPa·s after conversion into nanoemulgel. This reduction may reflect interactions between the dispersed phase, ZnO particles, and the

Carbopol network, although confirmation requires rheological and microstructural analyses. Carbopol concentration and formulation composition are known to influence nanoemulgel viscosity and rheological behavior. The low viscosity may also affect physical stability and phase separation during storage. Although the viscosity was below the 2,000–50,000 cP range specified in SNI 16-4399-1996, the formulation achieved an SPF of 17.47, indicating measurable photoprotective activity. Similar ZnO–Carbopol nanoemulgels have been evaluated based on viscosity, SPF, and physical stability [21].

Particle Size and Polydispersity Index

DLS analysis showed a *Z-average* particle size of 77.85 nm for the selected nanoemulsion (5% ZnO, 15 min stirring), increasing to 147.77 nm after conversion into nanoemulgel. Both remained within the nanoscale range, indicating retention of nanoscale dispersion after incorporation into the Carbopol matrix. The increase in hydrodynamic diameter may reflect changes in the dispersion environment and restricted droplet mobility within the gel network, as reported for Carbopol-based nanoemulgels [28]. The PDI increased from 0.0270 to 0.2554 but remained below 0.30, indicating a relatively narrow particle-size distribution. DLS provides hydrodynamic size and PDI measurements at the time of analysis but does not by itself establish long-term physical stability [29]. Despite the acceptable particle size and PDI, sedimentation and phase separation were observed in formulations with higher ZnO concentrations during storage. Therefore, further optimization of the gel matrix and dispersion system is required to improve physical stability while maintaining nanoscale characteristics and photoprotective performance.

Conclusion

ZnO concentration and stirring time significantly influenced the photoprotective and physicochemical characteristics of the green tea (*Camellia sinensis*) leaf extract nanoemulgel. The formulation containing 5% ZnO and stirred for 15 min achieved the highest SPF (17.47) and maintained a nanoscale particle size (147.77 nm). However, its physical stability was limited by phase separation during storage, whereas formulations containing 1–2% ZnO showed better physical homogeneity. Therefore, ZnO concentration and processing conditions are critical formulation parameters, but maximizing SPF alone is insufficient to define the optimal formulation. Further optimization of the ZnO dispersion and gel matrix is required to improve physical stability while maintaining the desired nanoscale characteristics and photoprotective performance.

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