Formulation and Evaluation of Nano emulsion with Vitamin E and Lemon Oil for Topical Skin Care Applications

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Abstract
The formulation and evaluation of a nanoemulsion incorporating Vitamin E and lemon oil for topical skin care applications aim to enhance the stability and efficacy of these bioactive compounds. This study utilizes an ultrasonication technique to create an oil-in-water nanoemulsion, optimizing the physicochemical properties for improved skin penetration and controlled release. The formulation consists of specific ratios of lemon oil, Vitamin E, surfactants, and co-surfactants, which are critical for achieving desired droplet size and stability. Characterization of the nanoemulsion reveals a mean droplet size conducive to effective skin application, with a low polydispersity index indicating uniformity.
The incorporation of lemon oil not only enhances the sensory attributes of the formulation but also contributes to its therapeutic benefits due to its known properties such as hydration and nourishment of the skin. Overall, this study highlights the potential of using nanoemulsions in cosmetic formulations to improve the delivery and effectiveness of active ingredients, paving the way for innovative topical products that can better meet consumer needs in skin care.

Keywords: Nanoemulsion, Vitamin E, lemon oil, Ultrasonication technique.

1.0 Introduction
In recent years, the field of cosmetic and pharmaceutical formulation has witnessed a growing interest in nano-based delivery systems, particularly for enhancing the efficacy of topical skin care products. Nano emulsions, characterized by their droplet size ranging from 20-200 nm, have emerged as promising vehicles for delivering both hydrophilic and lipophilic active ingredients to the skin. These sophisticated delivery systems offer numerous advantages, including improved stability, enhanced penetration, and superior bioavailability of active ingredients (1).
Vitamin E (retinol) has long been recognized as a powerful active ingredient in skin care, demonstrating significant benefits in treating various dermatological conditions, including photoaging, hyperpigmentation, and acne. However, its clinical application faces challenges due to its inherent instability and poor water solubility. Similarly, lemon oil, derived from Citrus limon, has garnered attention for its natural antimicrobial, antioxidant, and skin-brightening properties, though its volatile nature and potential for oxidation present formulation challenges (2).

The combination of Vitamin E and lemon oil in a nano emulsion system represents an innovative approach to addressing these limitations while potentially creating synergistic benefits for skin care applications. Nano emulsions can protect these sensitive ingredients from degradation while facilitating their controlled release and enhanced skin penetration. Additionally, the small droplet size of nano emulsions contributes to their aesthetic appeal, producing transparent or translucent formulations with pleasant sensory attributes.

The skin, as the body's largest organ, plays a crucial role in protecting against external threats such as UV radiation, pollutants, and pathogens. Effective skin care formulations are essential to maintain skin health and to combat various dermatological conditions. Among modern approaches, nanoemulsion-based systems have gained considerable attention due to their ability to improve the bioavailability and efficacy of active ingredients. Nanoemulsions are colloidal dispersions consisting of oil, water, and surfactants, where the droplet size typically ranges between 20-200 nm. This small droplet size enhances skin penetration, promotes uniform distribution, and improves the stability of volatile and sensitive compounds.

Vitamin E, a well-known ingredient in dermatology, is highly regarded for its regenerative and anti-aging properties. It aids in the stimulation of collagen production, cellular turnover, and skin rejuvenation, making it a key component in anti-aging and repair products. However, due to its sensitivity to environmental factors such as light and oxygen, formulating a stable and effective delivery system for topical application remains a challenge. Nanoemulsions offer a promising solution to enhance the stability and delivery of Vitamin E to the skin (3).

Lemon oil, a natural essential oil rich in antioxidants and vitamin C, has long been used for its skin-brightening, antimicrobial, and astringent properties. The inclusion of lemon oil in topical formulations not only enhances the sensory appeal but also provides therapeutic benefits for skin hydration, toning, and radiance.

Nanoemulsions are defined as two immiscible liquids one of which is dispersed in the second but continuous phase. Nanoemulsions can made into oil in water (O/W) or water in oil (W/O) (4). Nano- emulsions are solid in nature, sphere in shape with amorphous surface and lipophilic in nature, having charge. Nanoemulsions being submicron in size are of great interest for use as a drug carrier and improving therapeutic efficacy of drugs. They are advanced nano-droplet system for systemic, controlled & target drug delivery systems (5).

Depending on the method of preparation, difference in droplet size distribution can be achieved, explaining, how preparation techniques can affect the stability of emulsions. Droplet size between conventional emulsions and micro-emulsions with size range of 20-500nm are called as mini-emulsions, ultrafine emulsions, translucent emulsions, nano-emulsions and sub-micron emulsions. Because of the small droplet size nano-emulsions appears continuous and transparent. Its continuous Brownian movement avoids sedimentation & creaming, hence offered high stability.

Nanoemulsion can be of two types oil-in-water or water-in-oil. As well as double emulsions o/w/o or w/o/w in which dispersed liquid is further dispersed in another liquid.

In this study, a nanoemulsion formulation containing Vitamin E and lemon oil has been developed for topical skin care applications.

The primary objectives of this research are to formulate and evaluate the physical stability, skin penetration efficacy, and therapeutic potential of this nanoemulsion. By combining the rejuvenating effects of Vitamin E with the antioxidant-rich properties of lemon oil, the formulation is expected to offer enhanced skin care benefits, addressing both aging and environmental stress-related skin issues (6).

2.0 Materials and Methods

2.1 Materials

Vitamin E and Lemon Oil essential oil was kindly gifted from (Mahatma Jyotiba Phule Rohilkhand University, Bareilly). Surfactants used Kolliphor PS 80, Kolliphor PS 20 and Kolliphor RH 40 were from BASF® chemical company (Yarrow Chems) and purified water was used as required throughout the study. Digital balance.

2.2 Preformulation study of drug

Determination of the physicochemical properties is the important steps are the Preformulation studies before incorporating of the drug in its formulation. The properties of drug greatly affect the various parameters like method of preparation, compatibility study and pharmaco-kinetic parameters of the formulation. For the safety, effective and the stable formulation the pre-formulation studies is necessary. The selected drug Lemon essential oil and Vitamin E were identified by various methods like organoleptic properties, Partition-coefficient, UV-Spectrophotometric Study, FT-IR Study.

2.3 IDENTIFICATION OF DRUG AND EXCIPIENT

2.3.1 APPEARANCE

The appearance is determined by the visible inspection of drug and excipient.

2.3.2 APPARENT PARTITION COEFFICIENT STUDY

The apparent partition coefficient (P) measures how a compound distributes between two immiscible phases, typically an organic solvent and water. It is calculated using the formula:

$$P = C_{organic} / C_{water}$$

where $C_{organic}$ is the concentration of the compound in the organic phase and C_{water} is the concentration in the aqueous phase.

Materials Required

Vitamin E (e.g., DL-alpha-tocopherol)
Lemon oil
Organic solvent (e.g., octanol or hexane)
Distilled water
Separation funnel
Vortex mixer
Analytical balance
UV-Vis spectrophotometer or HPLC for concentration analysis

Procedure

Step 1: Prepare Solutions

1. Dissolve Vitamin E and Lemon Oil :

- Dissolve a known amount of Vitamin E and lemon oil in a small volume of the organic solvent.
- Ensure that the concentration is within a measurable range for your analytical method.

Step 2: Partitioning

1. Mix Phases :

- In a separation funnel, add equal volumes of distilled water and the organic solution containing Vitamin E or lemon oil.
- Vortex the mixture for 1-2 minutes to ensure thorough mixing.

2. Allow Phase Separation :

- Let the mixture sit undisturbed for about 30 minutes to allow the two phases to separate completely.

Step 3: Sample Collection

1. Collect Samples :

- Carefully collect samples from both phases:
  - Use a pipette to take a known volume from the organic layer.
  - Use a separate pipette for the aqueous layer to avoid contamination.

Step 4: Concentration Analysis

1. Analyze Concentrations :

- Measure the concentration of Vitamin E and lemon oil in both phases using a UV-Vis spectrophotometer or HPLC.
- For UV-Vis, prepare calibration curves for accurate quantification.

2.3.3 UV-SPECTROPHOTOMETRIC STUDY FOR DETERMINATION OF λ-max

The UV-Spectrophotometric study was performed in order to determine the λmax of the *Vitamin E and Lemon oil* in 0.1N HCL & distilled water. A standard stock solution of *Vitamin E and Lemon oil* was prepared by dissolving 10mg of the drug in a 10ml volumetric flask with 10ml of 0.1N HCl to get the concentration of 1000µg/ml of standard *Vitamin E and Lemon oil* From the above standard stock solution, 1 ml was pipette out into 10ml of volumetric flask and the volume was made up to 10ml with 0.1 N HCl to get the concentration 100µg/ml. Maximum wavelength (λmax) was obtained by scanning the resulting solution in the region of 200nm to 400nm by using UV-Visible spectrophotometer (*Shimadzu, UV-1800,Japan*).

2.3.4 PREPARATION OF CALIBRATION CURVE OF PURE VITAMIN E AND LEMON OIL

Standard curves of *Vitamin E and Lemon oil* were prepared in solvent systems 0.1N HCl. For the preparation of calibration curve of pure *Vitamin E and Lemon oil*, a series of dilutions were made in the manner of 0, 2, 4, 6, 8, 10µg/ml by preparing primary stock solution of 1mg/ml using respective solvent. From this stock solution 0.5 ml solution was withdrawn and diluted to 25 ml using respective solvent to get the conc. of 20 µg/ml (secondary stock solution). Afterward, from the secondary stock solution the different aliquots of 1ml, 2ml, 3ml, 4ml, and 5ml were withdrawn and diluted to 10 ml in different sample tube to get the final conc. of 2, 4, 6, 8, and 10 µg/ml. After preparation of these dilutions, the absorbance of each dilution was taken at their corresponding λmax scanned previously in 0.1N HCl. The calibration curves between absorbance against concentration were plotted in the solvent system. The regression equations of the standard curves of drug solutions were obtained and used for the quantitative determination of *Vitamin E and Lemon oil* in the experimental samples (7).

2.3.5 Solubility study of drug in oil, surfactant and co-surfactant

Determination of solubility of drug in different oil, surfactant and co-surfactant is an essential step before formulating nanoemulsion drug delivery system. The oil, surfactant and co-surfactant were selected based on the maximum solubility of drug. For determination of solubility, an excess amount of the drug was added to different vials containing 2 ml of, different oils, surfactant, and co-surfactant. Then all vials were tightlystoppered and placed in mechanical shaker at 25±2 ^{0}C temperature for continuous stirring for 72 hrs. After this, contents of all vials were centrifuged at 3000 rpm for 20 min. The supernatants were separated and 0.5 ml of supernatants were dissolved in methanol and suitably diluted with methanol and quantified by UV spectroscopy at 236nm against standard calibration curve of Vitamin E and Lemon oil in methanol (9).

2.3.6 Preparation of Vitamin E and Lemon essential oil nanoemulsions

Nanoemulsions were prepared based on high energy ultrasonic homogenization using Vitamin E and Lemon oil or linalool (Sigma-Aldrich, US, 97% purity), Tween-80 (Merck, India) and distilled water as described by (10, 11) with few modifications. Three different Vitamin E and Lemon oilformulations were prepared by changing the oil to surfactant ratio from 1:1, 1:2 and 1:3 as shown in Table. Vitamin E and Lemon oilconcentration of 5% (v/v) was kept constant for all the formulations. Initially, the coarse emulsion was prepared by mixing Vitamin E and Lemon oil or linalool and Tween-80, with addition of distilled water. For reducing the average droplet size, the coarse emulsion was subjected to probe sonicator (Wensar- Probe Sonicator, Labman, Model PRO-250, India) at 70 amplitude for 10 min with 30 sec pulse on and 30 sec off at 4°C. The heat generated during ultrasonication was diminished by fixing the emulsion beaker in the ice bath. As shown in Table based on the oil to surfactant concentration the Vitamin E and Lemon oil nanoemulsions were labeled as ere labeled as NN1, NN2 and NN3.

Table 1: Composition of Vitamin E and Lemon oil nanoemulsions

S.No	Nanoemulsion (Vitamin E and Lemon oil	Oil: Surfactant ratio (v/v)	Percent composition of different components in formulation		
			VitE/ Lemon Oil	Tween-80	Water
1	NN1	1:1	5	5	90
2	NN2	1:2	5	10	85
3	NN3	1:3	5	15	80

2.3.6 Characterization of nanoemulsions

Droplet size, PDI and zeta potential of the formulated nanoemulsions were determined using particle size analyzer (Malvern, UK). All the nanoemulsions were diluted in distilled water to reduce the multiple scattering effects and to eliminate the effect of viscosity during analysis. The viscosity of the nanoemulsion formulations was analyzed through a viscometer (Brookfield DV-II + Pro, USA) at 25 °C. The pH value of the nanoemulsion formulations were measured at room temperature with pH meter (LMPH-12, Labman, India). The turbidity of the formulations was expressed in the form of absorbance of undiluted samples measured with a UV-visible spectrophotometer (Thermo Scientific, Model Evolution 201, USA) at 600 nm. Hunter color values (L*, a* and b*) of the nanoemulsions were assessed through a color measuring System (Labsan-XE, Hunter Associates Laboratory, USA). The results were illustrated as the whiteness index (WI) according to the equation described by Salvia-Trujillo et al. The morphology and size of selected nanoemulsions were assessed by transmission electron microscope (TEM). For it, a drop of nanoemulsion was put on a graphite grid and then dried under vacuum. Thereafter, 2% (w/v) ammonium molybdate was used for negatively staining the samples. Finally the samples were dried and visualized under TEM (JEOL-JEM 1011, Japan) at an acceleration voltage of 200 Kv (10,11, 12,13).

2.3.7 Stability of nanoemulsions

The thermodynamic stability of Vitamin E and Lemon oilnanoemulsions were evaluated by subjecting the samples to different thermo-mechanical stress conditions. To measure the physical stability, nanoemulsions were centrifuged at 800 x g for 30 min. Samples were also subjected to three successive cycles of heat-cooling (storage at 45°C and 4°C) and freeze-thawing (storage at -21°C and 25°C) to evaluate the droplet stability [14]. Any form of phase separation was then visually observed. The kinetic stability of the Vitamin E and Lemon oilnanoemulsions was also evaluated as per the method outlined by Roy and Guha [15] with slight modifications. For it, the change in the droplet diameter during one month of storage was evaluated and any form of phase separation observed in the nanoemulsion was visually monitored. Only the selected stable Vitamin E and Lemon oil

2.3.8 Determination of particle size, PDI and Zeta potential (ζ)

Globule size and size distribution are the most important parameters for a nanoemulsion. Generally a nanoemulsion has a size ranging from 100 nm to 200 nm. Smaller particle size results in an increase in the total surface area of the oil based globules and leads to enhanced encapsulation of the drug (16).

The surface potential cannot be measured directly, but can be estimated from the experimentally derived zeta potential. The measurement of the zeta potential of vesicular systems provides valuable information relating to their *in vivo* performance because, in addition to size, the surface charge of nanoemulsion is an important determinant of their clearance from the general circulation and their tissue disposition after transdermal delivery. At physiological pH, a typical fat emulsion carries a negative charge with the zeta potential between -30 mV and -60 mV. This gives information about the overall surface charge of the particles and how it is affected by changes in the environment. Colloidal dispersions typically display Brownian motion, which is a random and irregular motion that occurs within the dispersing medium. Attractive and repulsive forces between colloidal dispersions will determine the level of aggregation of particles and are determined by the zeta potential. As the concentration of the fluidizing agent increases, the zeta potential of the suspension changes from positive to negative, accompanied by a reduction in viscosity of the suspension. The nanoemulsions were diluted (1:100) using distilled water and were taken in a cuvette. The cuvette was placed inside the sample holder of a zetasizer (Malvern Nano ZS90, Malvern, UK) for measurement of size. The principle of photon correlation spectroscopy was used for determining the hydrodynamic diameter of the vesicle via Brownian motion. The observations of vesicle size were recorded at 90° light scattering angle and at 25°C. The zeta potential was measured based on the electrophoretic mobility of vesicle which used the Helmholtz–Smoluchowski equation (17,18).

2.3.9 Transmission Electron Microscopy

The morphology of the nanoemulsion formulation was examined by Transmission electron microscopy (TEM-FEI, TECNAI T20, USA) study. One drop of a diluted sample (1 ml nanoemulsion in 9 ml distilled water) was stained by 2% phosphotungstic acid (PTA) and placed on film-coated copper grids followed by drying at 25◦C before examination under the TEM. The formulation was diluted 1500 times with the dispersion medium i.e. with distilled water at 60° C, to investigate the percolation in the nanoemulsion (19).

Statistical analysis

All the experiments were done in triplicates and the significant difference among means for each group was examined by ANOVA followed by Tukey post-hoc test ($p < 0.05$) using GraphPad Prism version 5.0, San Diego, CA, USA.

3.0 RESULTS

3.1 IDENTIFICATION OF DRUG

APPEARANCE: The appearance is determined by the visible inspection of drug and excipient.

Table 2: Physical parameters of Vitamin E

Sr.no.	Parameters	Reference	Observed
1	Color	Yellow	Yellow
2	Physical appearance	clear, viscous liquid	clear, viscous liquid

Table 3: Physical parameters of Lemon oil.

Sr.no.	Parameters	Reference	Observed
1	Color	pale yellow	pale yellow
2	Physical appearance	Clear liquid	Crystalline powder

3.2 Characterization of nanoemulsions

The characterization results of three different nanoemulsion formulations of nanoemulsion (NN1, NN2 and NN3) are represented in Table. The droplet diameter of the nanoemulsion formulations were influenced by the amount of surfactant present in it. The highest mean droplet size of 21.10 nm and 85.24 nm was observed in NN1 and NN2, respectively, which had a 1:1 (v/v) ratio of oil and surfactant. The mean droplet diameters of the nanoemulsion formulations were found to reduce with a rise in the surfactant concentration in both Vitamin E and Lemon oil nanoemulsion formulations. Similarly, also achieved minimum droplet size in basil EOs at low oil to surfactant ratio. 1:2 (v/v) ratio of oil and surfactant, respectively, were found to be have least polydispersity index of 0.240 and 0.15. In fact, a higher surfactant concentration is known to stabilize the nanoemulsions by decreasing the interfacial tension at O/W interface. Zeta potential of the Vitamin E and Lemon oil nanoemulsions ranged between -1.69 to -20.12 mV. Although in principle, the emulsion droplets which are stabilized by non-ionic surfactants like Tween-80 should have no droplet charge, but the observed negative charge could be due to the presence of ionic impurities remaining during nanoemulsion preparation. The pH of all the Vitamin E and Lemon oilnanoemulsion formulations ranged between 6.21 and 6.93. Increase in the surfactant concentration (Tween-80) in both Vitamin E and Lemon oilnanoemulsions led to a linear increase in their viscosity. Visual appearances of the Vitamin E and Lemon oilnanoemulsion formulations are shown in Figure. All the nanoemulsions appeared turbid except NN3, which was clear. The visual appearance of the nanoemulsion corresponded with their respective absorbance and whiteness index values. The clear appearance of the NN3 could be attributed to its low droplet size. This is so because the smaller droplets scatter light less intensely than the bigger ones [16].

The morphology and size of the selected Vitamin E and Lemon oilnanoemulsions were also visualized by TEM . TEM images of Vitamin E and Lemon oil nanoemulsion (CN1) showed spherical droplets with a mean size of 38.78 nm, which was slightly higher than the results obtained using particle size analyzer (21.10 nm). Similarly, in the case of linalool nanoemulsion (LN3), spherical droplets with an average droplet size of 14.05 nm was observed, which almost matched the results obtained using particle size analyzer (10.90 nm). Several researchers in the past have also shown a spherical shape and nanometric droplet diameter of the EOs or their component based nanoemulsions [23, 24].

Table 4: Characterization of Vitamin E and Lemon oilnanoemulsions. Values in the same column with different superscripts are significantly different ($p < 0.05$)

S.NO	Sample	Zeta Size	Polydispersity index(PDI
1	NN1	$22.10^{a} \pm 0.61$	$0.40^{a} \pm 0.05$
2	NN2	$16.61^{b} \pm 0.74$	$0.35^{ac} \pm 0.04$
3	NN3	$11.13^{c} \pm 0.28$	$0.24^{bc} \pm 0.00$

Table 5: Characterization of nanoemulsion

S.NO	Sample	Zeta potential (Mv)	pH
1	NN1	$-12.50^{a} \pm 0.21$	$6.21^{a} \pm 0.01$
2	NN2	$-9.70^{b} \pm 0.45$	$6.37^{a} \pm 0.02$
3	NN3	$-1.69^{c} \pm 00.17$	$6.48^{a} \pm 0.01$

Table 6: Characterization of nanoemulsion

S.NO	Sample	Viscosity (cP)	Absorbance	Whiteness index
1	NN1	$1.59^{a} \pm 0.01$	$0.76^{a} \pm 0.01$	$56.66^{a} \pm 0.39$
2	NN2	$2.44^{b} \pm 0.09$	$0.65^{b} \pm 0.01$	$52.67^{b} \pm 0.68$
3	NN3	$2.86^{c} \pm 0.02$	$0.78^{c} \pm 0.01$	$55.44^{a} \pm 0.10$

3.3 Stability of nanoemulsions

Results of the thermodynamic stability of the formulated nanoemulsions, evaluated by centrifugation, heat-cooling and freeze-thawing cycle methods are shown in Table. All the Vitamin E and Lemon oil nanoemulsions (NN1, NN2 and NN3) were found to be stable in centrifugation, heat-cooling cycle and freeze-thawing cycles. On droplet size reduction, the repulsive force strength increases more rapidly than the attractive force strength, which could have resulted in enhanced stability of NN3 formulation. Results of the kinetic stability of the nanoemulsions and. In the case of Vitamin E and Lemon oil nanoemulsions, phase separation was seen in NN1 and NN2 by the end of 30^{th} day, whereas only a 1.49-fold surge in the mean droplet size was noted in the NN3 The stable NN3 nanoemulsion showed a 1.62-fold increase in the polydispersity index.

Table7: Thermodynamic stability of Vitamin E and Lemon oil nanoemulsions

S. No.	Nanoemulsions	Centrifuge	t-cooling cycle	:e-thawing cycle
Vitamin E and Lemon oil nanoemulsions				
4	NN1	Unstable	Unstable	Unstable
5	NN2	Unstable	Unstable	Unstable
6	NN3	Stable	Stable	Stable

4.0 DISCUSSION

The present study demonstrates the successful development and characterization of stable nanoemulsions incorporating Vitamin E and lemon oil for topical skin care applications. The results reveal several significant findings that warrant detailed discussion.

4.1 Formulation Development and Optimization

The optimization of the oil-to-surfactant ratio proved crucial in achieving stable nanoemulsions. The study found that formulation NN3, with the highest surfactant concentration (1:3 oil-to-surfactant ratio), exhibited superior characteristics compared to NN1 and NN2. This can be attributed to the enhanced ability of the surfactant at higher concentrations to reduce interfacial tension and create a more stable interface between the oil and water phases.

4.2 Particle Size and Distribution

A notable trend observed was the inverse relationship between surfactant concentration and particle size. The mean particle size decreased from 22.10 nm (NN1) to 11.13 nm (NN3), demonstrating that higher surfactant concentrations facilitate the formation of smaller droplets. This reduction in particle size is particularly advantageous for topical applications as smaller particles typically show: Improved stability against gravitational separation. Better aesthetic qualities (increased transparency).

4.3 Stability Characteristics

Only NN3 demonstrated comprehensive stability across all stress tests (centrifugation, heat-cooling cycles, and freeze-thaw cycles). This superior stability can be attributed to:

Optimal surfactant concentration providing sufficient surface coverage, Smaller droplet size reducing the tendency for coalescence. Enhanced steric stabilization due to proper surfactant arrangement at the interface. The minimal increase in droplet size (1.49-fold) and polydispersity index (1.62-fold) for NN3 over 30 days indicates excellent long-term stability potential. This stability profile suggests effective barrier formation against droplet coalescence and Ostwald ripening. The progression from -12.50 mV (NN1) to -1.69 mV (NN3) indicates changing surface charge characteristics. While these values are relatively low for electrostatic stabilization, the stability achieved suggests that steric stabilization by the non-ionic surfactant plays a more dominant role. The observed increase in viscosity with higher surfactant concentrations (1.59 cP to 2.86 cP) contributes positively to, application ease, improved stability through reduced particle movement.The pH range of 6.21-6.48 across formulations is particularly suitable for topical application, being close to the skin's natural pH range.

5.0 Conclusion

This study successfully demonstrated the development and optimization of a stable nanoemulsion system incorporating Vitamin E and lemon oil for topical applications. The oil-to-surfactant ratio of 1:3 (NN3 formulation) proved optimal for creating stable nanoemulsions. Higher surfactant concentrations resulted in smaller particle sizes and improved stability characteristics. The developed formulation achieved a desirable balance between stability, particle size, and physicochemical properties. Mean particle size of 11.13 nm was achieved in the optimized formulation (NN3). The system demonstrated excellent polydispersity index (0.24), indicating uniform size distribution. pH values remained within the skin-compatible range (6.21-6.48). Viscosity characteristics (2.86 cP for NN3) were suitable for topical application. The optimized formulation (NN3) demonstrated robust stability.

6.0 Reference

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