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Green Synthesis of Anti-Aging Gel Mask Contains Essential Oils Based Nanoemulsions

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Abstract

This study investigates the formulation and evaluation of eco-friendly nanoemulsion-based gels (GN1–GN6) that incorporate natural ingredients, specifically lavender oil and myrtle oil. These oils are recognized for their anti-inflammatory, antioxidant, antiseptic, and immune-boosting properties, making them ideal candidates for counteracting the effects of aging. The primary objective was to develop a stable and therapeutically effective topical gel contain environmentally friendly natural oils in nanoemulsion form to be promising product against photoaging and chronological aging. Utilizing a microwave-based method, a pseudoternary phase plot was created, leading to the assessment of six distinct nanoemulsion formulations (NE1–NE6) through thermodynamic and characterization processes that were used to prepare nanoemulsion-based gels (GN1–GN6). The results revealed that all nanoemulsion formulations exhibited favorable surface charge, low polydispersity indices, robust thermodynamic stability, and nanosized particle dimensions. Additionally, the evaluation of the gel formulations (GN1–GN6) demonstrated stable organoleptic properties, suitable pH and spreadability values, acceptable viscosity, no skin irritation and good biological membrane permeation ability. The therapeutic efficacy of the various chemical constituents within the essential oil-based nanoemulsions, combined with the positive evaluation results of the nanoparticle-based gels, positions these formulations as promising natural masks. They effectively restore skin health and protect against the adverse effects of aging and environmental stressors, particularly sun exposure.

1. Introduction

Throughout history, people have often changed their appearance to enhance their chances of success in various aspects of life, including society, combat, and relationships, or to boost their self-confidence. Various cultures have used different minerals, animals, plants, and chemicals to care for their skin and enhance their looks. However, it's important to note that beauty trends fluctuate over time, influenced by cultural and religious traditions. This is a natural part of life. The chemical industry aims to create high-quality products and frequently utilizes plants, which provide a continuous supply of raw materials typically considered safe and non-toxic. Skin

aging is part of the typical human aging mosaic, which manifests differently across various organs over time [1]. Signs of aging skin include the formation of wrinkles, loss of elasticity, and a rougher texture. This is the result of a continuous degeneration process caused by damage to cellular proteins and DNA. As a result, the skin becomes thinner, loses fat, and no longer appears smooth and plump. Veins and bones in the face become more prominent, and the healing of bruises, wounds, and scratches may become slower [2]. Two types of skin aging, age-dependent (chronological, intrinsic) and photoaging, or extrinsic aging. Age-dependent or intrinsic skin aging is influenced by various environmental factors, including smoking, repetitive facial expressions, gravity, and sun exposure. This type of aging results from the natural aging process over time. Hormonal fluctuations also play a crucial role, as levels of sex hormones, such as estrogen and progesterone, decline, particularly during menopause. This hormonal decrease can lead to symptoms such as dry skin, wrinkles, epidermal thinning, collagen loss, and reduced skin elasticity. Photoaging, or extrinsic aging, is primarily caused by prolonged exposure to ultraviolet (UV) radiation. It predominantly affects areas of the skin that are regularly exposed to the sun. Key factors in extrinsic aging include the extent of sun exposure and individual skin pigmentation. Chronic UV exposure damages the epidermis, thickens the stratum corneum, and promotes dysplasia with abnormal cell growth, decreases collagen levels, and leads to the degradation of elastic fibers. Both intrinsic and extrinsic aging share similar biological mechanisms, including telomere shortening, mitochondrial DNA damage, oxidative stress, gene mutations, and hormonal declines. These processes collectively contribute to the visible signs of skin aging [3, 4].

Cosmetics encompass a diverse array of products designed for application on the human body to enhance, maintain, or change one's appearance. They also serve to cleanse, color, condition, or protect the skin, hair, nails, lips, eyes, and teeth. Many cosmetic products aim to reduce oil production, treat acne, and minimize the appearance of wrinkles. Various formulations, such as sunscreens, anti-acne treatments, anti-wrinkle creams, and anti-aging products, are developed to address specific skin concerns using a mix of natural and synthetic ingredients [5, 6].

The longstanding interest in utilizing plants and their natural oils for treating various ailments has motivated researchers to integrate these ingredients into the pharmaceutical industry, particularly those with scientifically validated therapeutic benefits. Essential oils, which are extracted from various parts of plants, are widely used in cosmetics, fragrances, pharmaceuticals, and increasingly in agricultural applications. These volatile mixtures of organic compounds are primarily obtained through steam distillation, but can also be extracted via methods such as cold pressing, solvent extraction, or supercritical carbon dioxide extraction [7, 8]. Essential oils are recognized for their potent aromas and complex compositions, often containing hundreds of chemical constituents, particularly terpenoids. Key components typically include oxygenated compounds such as esters, aliphatic alcohols, terpenes, aldehydes, ketones, oxides (including epoxides), and lactones. The effects of essential oils are diverse and largely influenced by their predominant components. Common characteristics of these oils include volatility, lipophilicity at 18°C, liquid state, and optical activity [9, 10]. Lavender oil, derived from the fresh flowers of Lavandula angustifolia, is widely recognized for its use in aromatherapy, particularly in Mediterranean regions. It is one of the few essential oils that can be applied directly to the skin. When used in compresses, lavender oil promotes faster healing of wounds, burns, and insect bites, thanks to its antiseptic and anti-inflammatory properties. In preventive healthcare, it is primarily valued for its sedative, hypnotic, and antidepressant effects. Baths or massages with lavender oil can support the treatment of depression, anxiety, and insomnia, while also helping to reduce tension and stress. Additionally, its analgesic qualities make it beneficial for menstrual discomfort, rheumatic pain, muscular aches, and migraines. Topical application also enhances capillary dilation and blood flow, leading to increased circulation and localized warmth [11, 12].

Myrtle oil, extracted from the Myrtus communis L., is a prominent plant found along the Mediterranean coast, growing as a shrub or small tree. The berries, leaves, seeds, and essential oils of myrtle are packed with various nutrients and bioactive compounds that provide notable health benefits. Historically, myrtle has been utilized in traditional medicine to address a wide array of ailments, including gastrointestinal, urinary, and skin conditions. In contemporary times, myrtle oil is widely applied across the food, cosmetic, and pharmaceutical sectors [13, 14].

Lavender oil and myrtle oil are hydrophobic compounds, which makes them poorly soluble in water-based formulations like gels. To address this challenge, nanoemulsions provide a sophisticated delivery system by encapsulating these oils within an aqueous medium [15, 16]. This oil-in-water nanoemulsion is particularly suitable for topical applications. When mixed with a gelling agent, it creates a more effective formulation known

as a nanoemulsion-based gel [17-20]. This scientific study aims to develop a gel contain environmentally friendly natural oils in nanoemulsion form, focusing on creating a formulation that can reduce signs of aging.

2. Materials and Methods

2.1. Ethical Approval

This study was approved by the Ethical Statement Committee at Al-Mustaqbal University (Certificate No. Pha 7/2024, issued on June 15, 2024) and conducted in accordance with the National Committee for Research Ethics in Science and Technology (NENT) guidelines. All animal experiments followed ethical standards ensuring humane treatment and welfare.

2.2. Materials

Myrtle oil, and lavender oil were obtained from Nanjing Duly Biotech Co., Ltd in China. Triethanolamine was purchased from Avon Chem in the United Kingdom, while propylene glycol, Tween 80, Retinol and Carbopol 940 were acquired from Beijing Yibai Biotechnology Co., Ltd in China. All solvents and reagents used in this study were of analytical grade.

2.3. Methods

2.3.1. Preparation of Essential Oils Nanoemulsion and Construction of Pseudoternary Phase Diagrams Using the Microwave-Based Technique

In this study, the essential oils of myrtle and lavender were combined and subjected to a magnetic stirrer for 5 minutes to ensure thorough mixing. Concurrently, the hydrophilic components—distilled water, Tween 80, and propylene glycol—were also mixed using a magnetic stirrer for 5 minutes. Once these initial preparations were complete, the hydrophobic phase (essential oils) was blended with the hydrophilic phase. This mixture was then stirred at 1000 rpm for 5 minutes, following the specific proportions outlined in Table (1). The blend was subsequently heated briefly in a microwave device for 10-15 seconds, which facilitated the formation of the nanoemulsion. Following this heating step, the mixture was stirred again at 1000 rpm for approximately 15-20 seconds until the desired nanoemulsion characteristics were observed. To construct the pseudoternary phase diagram, the formulation consisted of three primary components: lavender oil and myrtle oil (mixed in a 3:1 ratio), a surfactant mixture of Tween 80 and propylene glycol (also in a 1:1 ratio), and distilled water. During the construction of the diagram, formulations were visually inspected for transparency while being subjected to magnetic stirring to determine phase boundaries. The phase diagram, as illustrated in Figure (1), was generated using Triplot V4 software version 4.1.2. The shaded area on the diagram represents the region where the nanoemulsion was successfully formed [17-20].

2.3.2. Preparation of Nanoemulsion-Based Gel (GN1–GN6) Formulations

The process of creating nanoemulsion-based gels began with the preparation of the carbomer 940 hydrogel. To achieve this, 0.5% (w/w) of the carbomer 940 gelling agent was carefully added to distilled water. This mixture was then subjected to vigorous stirring using an electric homogenizer to ensure a uniform dispersion. Once the gelling agent was thoroughly mixed, a few drops of triethanolamine were introduced to adjust the pH and complete the hydrogel formation. With the hydrogel ready, the next step involved the integration of the previously prepared nanoemulsion formulations (NE1-NE6). These nanoemulsions, each at a concentration of 35% (w/w), were gradually incorporated into the hydrogel base. This was done through continuous, slow stirring to ensure a homogeneous blend. The stirring process was maintained until clear, essential oil nanoemulsion-based gel formulations (GN1-GN6) were achieved. To preserve the integrity of these formulations, they were transferred into tightly sealed containers and stored at a controlled temperature of 25°C. This careful storage was crucial for maintaining the stability and efficacy of the nanoemulsion-based gels for subsequent experimental applications [17-20].

2.3.3. Studies of Thermodynamic Stability of Essential Oil Nanoemulsion (NE1-NE6) Formulations

The thermodynamic stability of eco-friendly essential oil based nanoemulsion formulations (NE1-NE6) was meticulously examined through a series of rigorous tests designed to ensure their robustness and efficacy. These tests aimed to verify the formulations' stability under various conditions, which is crucial for their potential application in diverse environments [17-20].

Table (1): Concentrations of eco-friendly nanoemulsion (NE1-NE6) and eco-friendly nanoemulsion -based gel (GN1-GN6) formulations.

Code	Lavender oil: Myrtle oil 3:1 (% w/w)	Tween 80 and propylene glycol mixture 1:1(%w/w)	Carbomer 940 (% w/w)	Distilled water up to (%w/w)
GN1	2	32	0.5	100
GN2	4	32	0.5	100
GN3	6	32	0.5	100
GN4	8	32	0.5	100
GN5	10	32	0.5	100
GN6	12	32	0.5	100

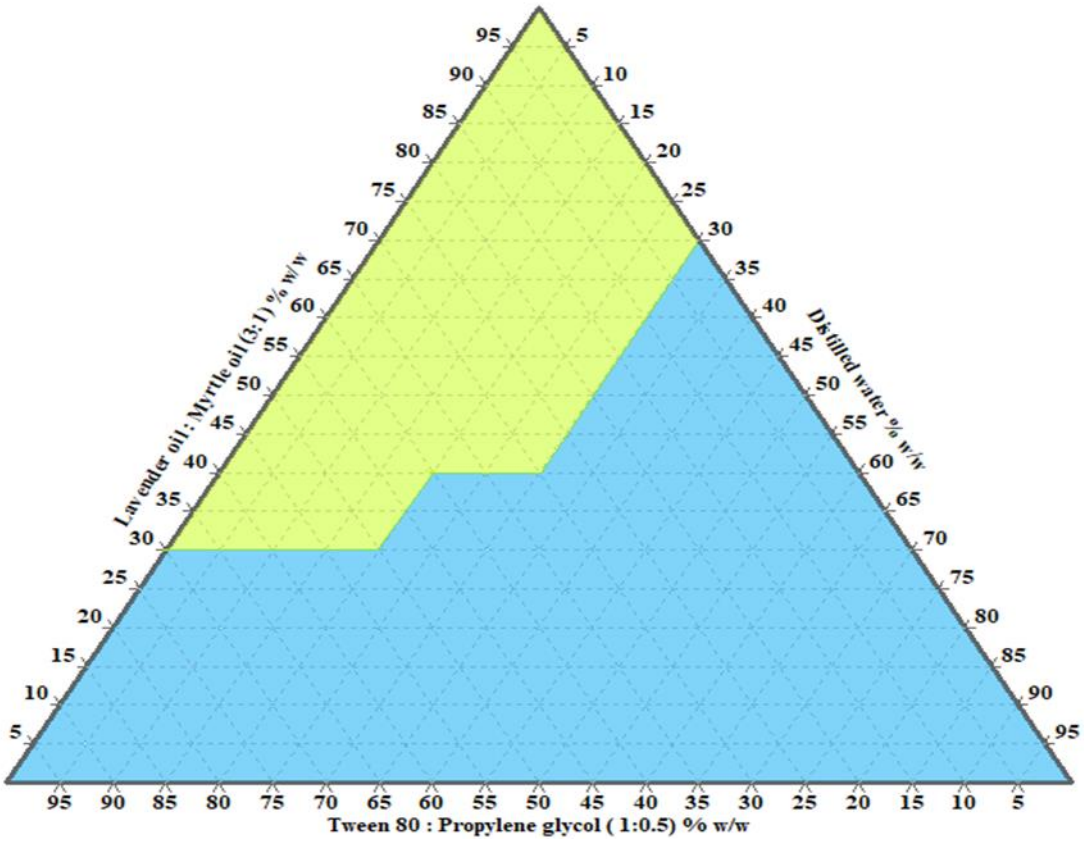

Figure (1): Pseudo ternary phase diagram was contained three components: lavender oil: myrtle oil (3:1), a surfactant mixture which are tween 80: propylene glycol (1:0.5) and distilled water.

2.3.3.1. Centrifugation Test

The first step in the stability assessment involved the centrifugation test. Here, the nanoemulsion formulations were subjected to a centrifugal force of 5000 rpm for 30 minutes. This process aimed to detect any signs of instability such as phase separation, creaming, or cracking. Observations made post-centrifugation were critical;

formulations that remained homogeneous without any visible separation or structural breakdown were considered stable. These formulations then advanced to further stability evaluations. To ensure accuracy and repeatability, each measurement was replicated three times, reinforcing the reliability of the results (17-20).

2.3.3.2. Heating and Cooling Test

Next, the heating and cooling test evaluated the formulations' stability under extreme temperature variations. The nanoemulsions were alternately held at 45°C and 0°C for at least 48 hours at each temperature. This procedure simulated the potential temperature fluctuations the formulations might encounter during storage or transport. Again, each measurement was duplicated thrice to ensure consistency. Formulations that maintained their integrity without phase separation or significant physical changes under these conditions were deemed stable [17-20].

2.3.3.3. Freeze-Thaw Test

The final step was the freeze-thaw test, which provided an accelerated stability evaluation by exposing the formulations to two extreme temperatures: -21°C and 21°C. Each cycle lasted for a minimum of 24 hours, simulating the stress conditions of freezing and thawing that might occur during shipping or seasonal changes. This test was also conducted in triplicate to confirm the reliability of the results. Formulations that withstood these temperature cycles without degradation or separation were confirmed to have robust thermodynamic stability.
These comprehensive stability tests demonstrated the resilience of the eco-friendly nanoemulsion formulations (NE1-NE6), affirming their potential for practical applications in various environmental conditions [17-20].

2.4. Characterization of Essential Oils Nanoemulsion (NE1-NE6) Formulations

The intricate process of characterizing essential oils nanoemulsion (NE1-NE6) formulations involved the precise determination of particle size, polydispersity index (PDI), and electrokinetic potential. These key parameters were analyzed using dynamic light scattering (DLS), a sophisticated technique facilitated by Horiba Instrument, Ltd. in Kyoto, Japan. Dynamic light scattering (DLS) operates by directing a laser beam through the sample, measuring the fluctuations in the intensity of scattered light caused by the Brownian motion of the dispersed lipid-based nanocarriers. The method's reliability was underscored by the consistent results obtained across three independent trials, ensuring the accuracy of the particle size and PDI measurements. Additionally, the electrokinetic or zeta potential, a crucial indicator of the stability of the nanoemulsions, was meticulously analyzed. A high zeta potential value typically signifies a stable nanoemulsion, preventing aggregation of the nanocarriers [17-20].

2.5. Atomic Force Microscopy

To further understand the morphology of the nanocarriers in the most optimized formulation, Atomic Force Microscopy (AFM) was employed. This analysis was conducted using an Angstrom Advanced Inc. AA3000 instrument from the USA, which offers a scanning range of 100 MV/s. In this method, two to three drops of the optimized nanoemulsion formulation were carefully placed on an experimental glass slide. After a stabilization period of three hours, the AFM provided detailed images and data, revealing the surface structure and morphology of the nanocarriers. These comprehensive characterization techniques not only confirmed the physical attributes of the nanoemulsion formulations but also provided insights into their stability and potential efficacy [20].

2.6. Evaluation of Essential Oil Nanoemulsion-Based Gel (GN1–GN6) Formulations

The comprehensive evaluation of essential oil nanoemulsion-based gel (GN1–GN6) formulations encompassed several critical assessments to ensure their stability, efficacy, and suitability for medicinal use.

2.6.1. Organoleptic Assessment

Organoleptic evaluation is fundamental in determining the physical stability of medicinal preparations. For the nanoemulsion-based gels (GN1–GN6) formulations, attributes such as color, odor, uniformity, and syneresis were meticulously examined at intervals of 0, 7, 14, 21, and 28 days. This rigorous assessment was conducted three times to guarantee the reliability of the results, ensuring that the formulations maintained their desired characteristics over time [17, 20].

2.6.2. Determination of pH

The pH level of the nanoemulsion-based gels was measured to predict their stability and compatibility with human skin. A ten-gram sample from each formulation was tested using a digital pH meter. Given that the ideal pH range

for human skin is between 4.5 and 6.5, it was crucial that the formulations needed to fall within this range. Each experiment was repeated three times to ensure accuracy and consistency in the results [17-20].

2.6.3. Measurement of Spreadability

The spreadability of the GN1-GN6 formulations was assessed using a straightforward yet effective method involving two glass slides, each measuring 10 × 2.5 cm. A wooden base containing 0.5 g of the gel was fixed to one slide, with the second slide placed on top. A weight of 25 g was then applied, and the time taken for the top slide to move 7.0 cm was recorded. This process, repeated three times, provided valuable data on the spreadability (S) of the gels, calculated using the formula [17-20]:

$$S = M \times L / T \ldots\ldots (1)$$

Where: S is the spreadability, M is the weight that triggers the movement of the first slide, L is the length of the slide, and T is the time taken for the two slides to separate completely.

2.6.4. Viscosity Measurement

Viscosity is a critical parameter in the development and evaluation of novel pharmaceutical formulations. The viscosity of the GN1-GN6 gels was measured at 25°C using a rotational digital viscometer equipped with a spindle number of 2. The samples were tested at various rotational speeds ranging from 0.1 to 60 rpm. This experiment, repeated three times, provided a comprehensive understanding of the viscosity profiles of the formulations, ensuring they met the desired consistency and stability criteria [17-20].

2.6.5. Skin Irritation Test

Approval for the skin irritation test was obtained from the Institutional Ethics Committee at the College of Pharmacy, University of Al-Mustaqbal, the approval code used for the study was pha 7/2024 on 15.6.2024. Fasted male rabbits, each weighing approximately 2.1 kg, were used to assess skin irritation. The rabbits were maintained on a standard diet with free access to food and water. Hair was removed from the skin surface, which was then divided into six areas, each approximately 4 cm² in size. A 0.5 g sample of nanoemulsion-based gels loaded with retinol (RGN1–RGN6) formulations was applied to each area twice daily. The test sites were monitored for any signs of erythema or edema at 1, 24, 48, and 72 hours after application. Skin sensitivity was graded on a scale of 0 to 3, where 0 indicated no reaction, 1 indicated slight patchy erythema, 2 indicated patchy erythema, and 3 indicated severe erythema with or without edema. [17-20].

2.6.6. Biological Membrane Permeation Study Using Retinol as Therapeutic Model

This study was conducted on fasted male rabbits weighing approximately 2.1 kg, following approval from the Institutional Ethics Committee at the College of Pharmacy, University of Al-Mustaqbal. The rabbits were euthanized, and the hairless abdominal skin was surgically excised. Subcutaneous fat was meticulously removed using cold normal saline. The excised skin was then cut into circular segments of 19.625 cm², which served as biological membranes. These segments were placed between the donor and receptor compartments of a Franz-type diffusion cell, with the receptor chamber containing 300 mL of phosphate-buffered saline (PBS) at pH 7.4. One-gram samples of RGN1–RGN6 formulations, each containing 0.3% retinol, were placed in the donor chamber of the Franz cell. Samples from the receptor chamber were collected at specified time intervals and analyzed for retinol content using a UV-visible spectrophotometer (Biobase Meihua Trading Co., Ltd) set at 345 nm. Each experiment was performed in triplicate. The permeability coefficients were calculated using Equation 2.

$$M = Peff\ S\ Cd\ tres \ldots\ldots (2)$$

Where: M = Amount of therapeutic agent absorbed, Peff = effective membrane permeability (cm/min), Cd = Initial concentration (donor concentration), tres = Residence time of the therapeutic agent at the absorption site, and S = Surface area available for absorption [20, 21].

2.7. Analytical Statistics:

The study's observations included the mean and standard deviation (SD) from the three experimental trials. Statistical analysis was performed using Excel, employing a one-way analysis of variance (ANOVA) with a significance threshold set at ($P \leq 0.05$). This rigorous statistical approach ensured the reliability and validity of the experimental data, providing a solid foundation for the formulation's stability and efficacy [17-20]. This detailed evaluation process ensured that the GN1–GN6 nanoemulsion-based gels were not only effective but also stable and compatible with human skin, making them suitable for further development and use in medicinal applications.

3. Results and Discussion

3.1. Characterization of Essential Oils Nanoemulsion (NE1-NE6) Formulations

The outcome of characterization of essential oils nanoemulsion (NE1-NE6) formulations as shown in Table (2), involved a comprehensive analysis. The study began with the construction of pseudo-ternary phase diagrams using three primary components: essential oils (in a 3:1 ratio), a surfactant mixture (1:0.5 ratio), and distilled water. This process was facilitated by a microwave-based method, ensuring rapid and accurate preparation of impurity-free samples. The phase diagrams revealed distinct regions, with the nanoemulsion of essential oils depicted as a shaded yellow area, and the coarse emulsions represented in blue, as shown in Figure (1). Six formulations (NE1, NE2, NE3, NE4, NE5, and NE6) were selected from this diagram for further characterization based on their promising properties. Myrtle oil and lavender oil were chosen due to their volatility and high flow rate, which are crucial for creating suitable nanocarriers within the hydrogel matrix. The particle sizes for NE1, NE2, NE3, NE4, NE5, and NE6 were determined to be 12.77 nm, 16.86 nm, 20.21 nm, 24.94 nm, 32.69 nm, and 42.02 nm, respectively, indicating their colloidal behavior. The increase in globule size correlated with the concentration of lavender oil and myrtle oil, likely due to the increased viscosity of the colloidal dispersion. This increased viscosity made the dispersed nanoparticles more resistant to breakdown into smaller particles during emulsification [17-21].

The PDI values ranged from 0.068 to 0.111, suggesting a highly uniform and constrained size distribution within the nanosystem. A lower PDI value indicates a more uniform particle size distribution, which is desirable for stability and consistency in nanoemulsion formulations [17, 20].

The mean electrokinetic potential values for the nanoemulsion formulations ranged from 11.6 to 37.42 mV. These values confirm the stability of the nanosystem, as a higher electrical charge on the surface of nanoparticles prevents clumping in solutions. The observed ZP values suggest good stability, which is essential for the practical application of these nanoemulsions [17, 20].

To evaluate the thermodynamic stability of the formulations, several rigorous tests were conducted, including cycles of freezing and thawing, as well as centrifugation. The results showed that all preparations demonstrated outstanding physical stability, indicating their robustness under varying conditions [17, 20].

An analysis of variance (ANOVA) was performed to explore the relationship between the oil content (independent variable) and the dependent variables (particle size, PDI, and ZP). The findings revealed a significant link, with a p-value ≤ 0.05, supporting the alternative hypothesis and rejecting the null hypothesis. This indicates that the oil content has a notable impact on the characteristics of the nanoemulsion formulations.

Table (2): Characterization results of eco-friendly nanoemulsion (NE1-NE6) formulations

Formulation Code	Globule size (nm)*	PDI*	Zeta potential*
NE1	12.77±0.82	0.068±0.001	11.616±0.202
NE2	16.86±0.65	0.077±0.001	16.383±0.447
NE3	20.21±0.461	0.086±0.001	21.686±0.482
NE4	24.94±0.593	0.097±0.0005	25.42±0.433
NE5	32.69±0.80	0.105±0.004	34.423±0.438
NE6	42.02±0.594	0.111±0.002	37.423±0.438

*Values are expressed as mean ± SD (n=3).

3.2. Atomic Force Microscopy Analysis

The AFM analysis of the NE6 nanoemulsion formulation provided valuable insights into its structural properties. The high-resolution images revealed that the particles are nanometer-sized, nearly spherical, and feature smooth surfaces, underscoring the precision in their preparation as shown in Figure (2). A key finding was the absence of particle aggregation, which indicates excellent physical stability and suggests that the formulation can maintain its efficacy and shelf-life. The uniformity in particle size and shape not only enhances the reliability of the product but also ensures consistent performance across different batches. Additionally, the smooth surfaces reduce friction, improving the nanoemulsion's effectiveness in drug delivery applications. The successful formulation, aided by appropriate surfactants and stabilizers, highlights the potential of using AFM as a critical tool for optimizing nanoemulsions, ultimately leading to superior therapeutic and cosmetic benefits [20].

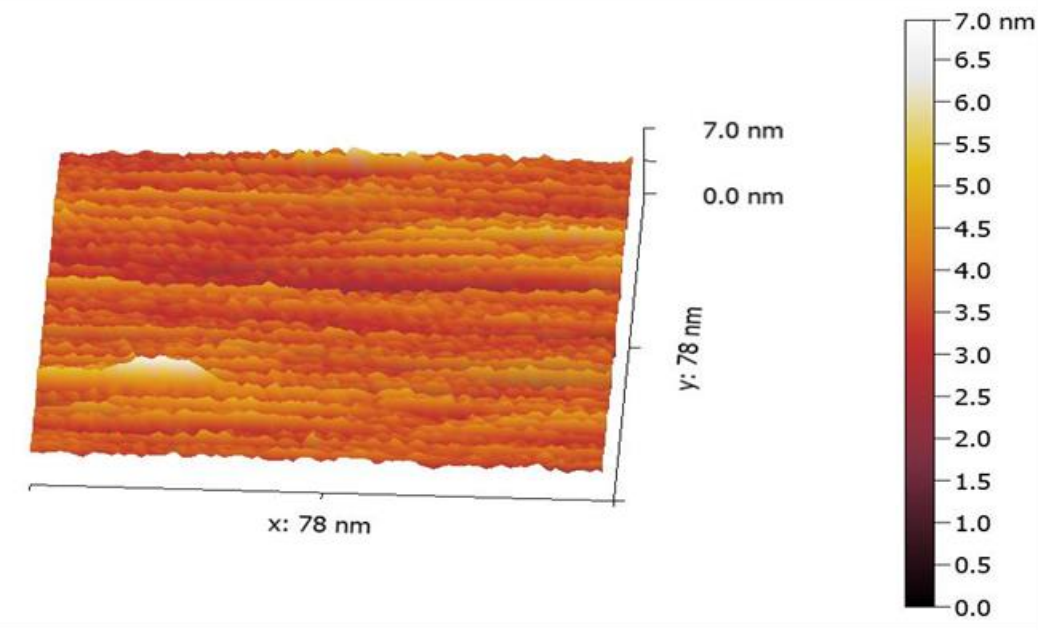

Figure (2): The AFM 3D image of eco-friendly nanoemulsion (NE6) formulations.

3.3. Evaluation of Eco-Friendly Nanoemulsion Based Gel (GN1–GN6) Formulations

3.3.1. Assessment of Organoleptic

The eco-friendly nanoemulsion-based gel formulations (GN1–GN6), a thorough organoleptic assessment was performed. This process, rooted in sensory analysis, relied exclusively on visual inspection and sensory observation. Each of the GN1-GN6 formulations exhibited a distinct and consistent aroma, a signature trait imparted by the essential oils used in their creation. This consistency in scent underscores the meticulous formulation process and the stable integration of essential oils within the gels.

Furthermore, the visual inspection revealed a notable absence of syneresis across all samples. This lack of syneresis—where a gel releases liquid—points to the high physical stability of the formulations. The stability is crucial for ensuring the longevity and effectiveness of the gels, making them reliable for extended use. The organoleptic evaluation, while seemingly simple, plays a critical role in the initial stages of product assessment. By ensuring that the formulations are visually appealing and stable, it sets a solid foundation for further, more rigorous testing and development. This step confirms that the GN1-GN6 gels not only meet sensory expectations but also possess the necessary physical integrity for practical applications [17-20].

3.3.2. Determination of pH

In evaluating the eco-friendly nanoemulsion-based gel formulations (GN1–GN6), determining the pH was a key focus to ensure patient satisfaction and product quality. Understanding the pH levels of these gels is essential, as they directly impact the comfort and safety of users. The pH values of the nanoemulsion-based gels (GN1-GN6) ranged from 5.2 to 6.2 as shown in Figure (3), reflecting a slightly acidic nature. This slight acidity aligns well

with the natural pH of the skin, which is important for maintaining skin health and preventing issues like dermatitis and allergies [17, 20].

The study also observed that increasing the concentration of essential oils, specifically lavender and myrtle oils, resulted in a minor increase in pH. This indicates that while the oils contribute beneficial properties to the formulations, they also influence the overall pH balance. Statistical analysis confirmed a significant correlation ($p \leq 0.05$) between the amount of essential oils and the pH levels, highlighting the oils' role as an independent parameter in the formulations.

Overall, the findings suggest that the pH levels of these gels are well-suited for patient use, ensuring comfort and minimizing the risk of adverse skin reactions. This pH evaluation underscores the meticulous approach taken in formulating these eco-friendly gels, prioritizing both efficacy and user safety [17-20].

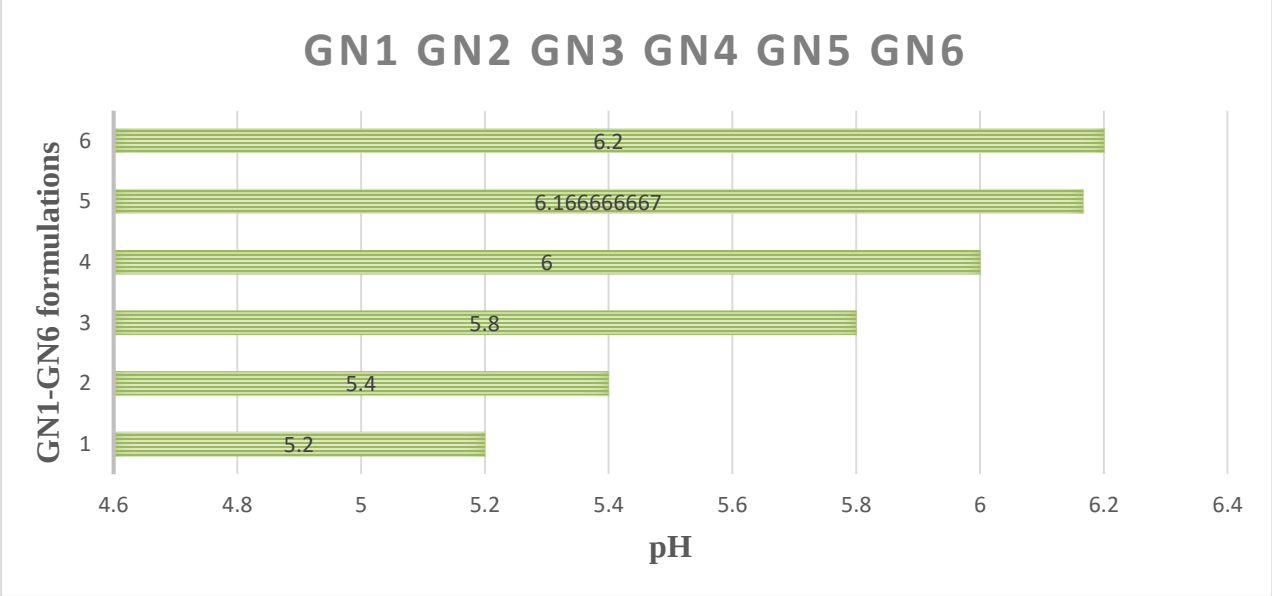

Figure (3): pH values of eco-friendly nanoemulsion-based gel (GN1–GN6) formulations.

3.3.3. The Measurement of Spreadability

In evaluating the eco-friendly nanoemulsion-based gel formulations (GN1–GN6), the measurement of spreadability was a key focus. The spreadability of these gel formulations ranged from 108.51 to 65.12 g*cm/sec as shown in Figure (4). This variation was directly influenced by the quantity of essential oils used. Increasing the amount of essential oil while maintaining constant levels of Tween 80, propylene glycol, and carbomer 940 resulted in decreased spreadability, primarily due to the higher viscosity of the gels.

These results suggest that the nanoemulsion-based gel formulations exhibit relatively low spreadability times, which can be advantageous for patient compliance during application. The gels' consistency ensures ease of use, enhancing the overall user experience. Statistical analysis revealed a significant relationship ($p \leq 0.05$) between the spreadability factor and the amount of essential oils, confirming the oils' role as an independent factor in determining the gels' spreadability. This highlights the careful balance needed in formulation to achieve optimal performance and user satisfaction [17-20].

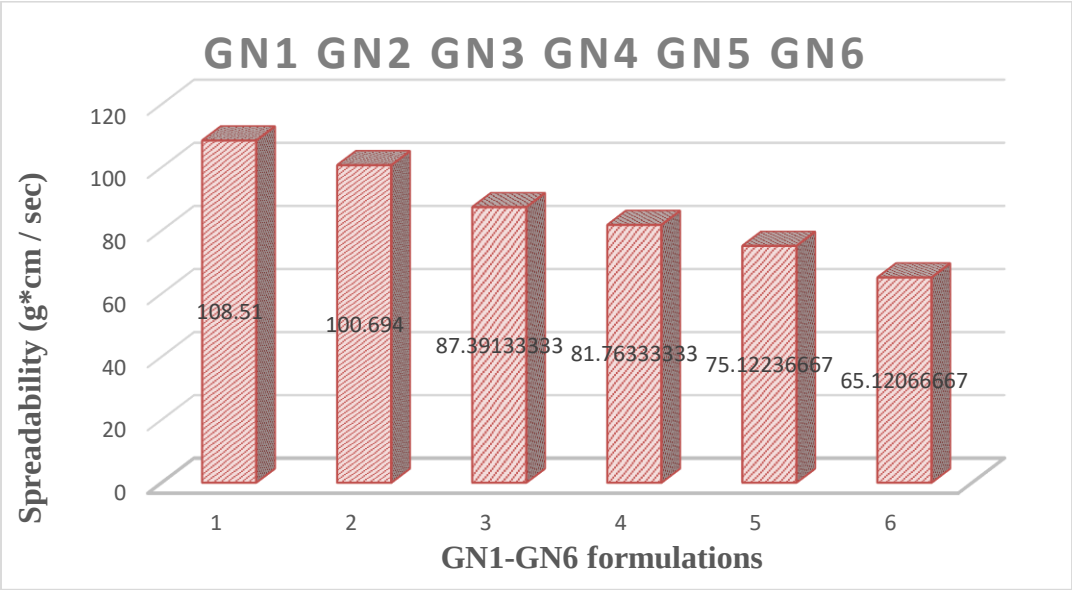

Figure (4): Spreadability values of eco-friendly nanoemulsion-based gel (GN1–GN6) formulations.

3.3.4. Viscosity Measurement

To determine the viscosity characteristics of the eco-friendly nanoemulsion-based gel formulations (GN1–GN6), a digital rotating viscometer from Biobase Meihua Trading Co., Ltd., equipped with a spindle number 2, was utilized. The findings, as presented in Table (3), demonstrated that increasing the amount of aromatic oil in a constant (% w/w) mixture of Tween 80 and propylene glycol resulted in heightened viscosity. At a rotational speed of 30 rpm, the viscosity values in mP.s units were recorded as follows: GN1=1121.56, GN2=1140.63, GN3=1167.5, GN4=1180.36, GN5=1200.3, and GN6=1220.4 mP.s as shown in Figure (5).

The observed rise in viscosity is attributed to a reduction in the volume of the aqueous phase, which makes the nanosystem more viscous, and a decrease in the continuous phase volume, which increases the resistance to flow within the colloidal dispersion system. This phenomenon occurs because the increased concentration of essential oils elevates the overall viscosity, leading to a denser gel structure.

Statistical analysis using ANOVA confirmed a significant association ($p \leq 0.05$) between the dependent variable, viscosity, and the independent factor, aromatic oil content. This correlation underscores the critical role of aromatic oil in influencing the rheological properties of the gel. These findings suggest that careful adjustment of the aromatic oil concentration is essential for optimizing the viscosity of nanoemulsion-based gels, ensuring their suitability for various pharmaceutical and cosmetic applications while maintaining the desired consistency and stability [17-20].

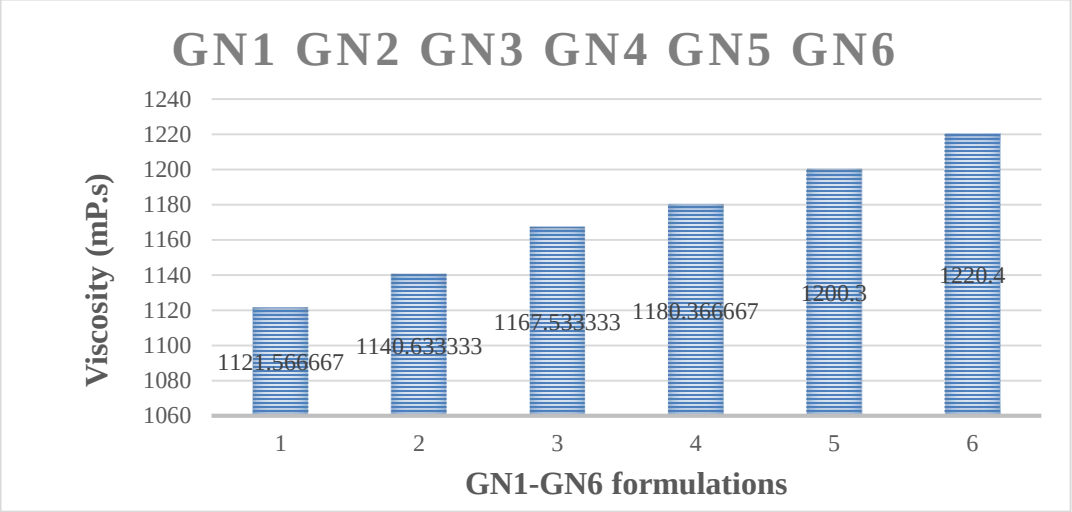

Figure (5): Viscosity values of eco-friendly nanoemulsion-based gel (GN1–GN6) formulations.

3.3.5. Skin Irritation Test

The skin irritation test was completed successfully, revealing no erythemic reactions at the application sites on the rabbit skin. This outcome confirms the safety of the retinol-loaded RGN1– RGN6 formulations when applied to biological membranes, as depicted in Figure (6). The absence of erythema in the skin irritation test suggests that the retinol-loaded GN1–GN6 formulations are well-tolerated and do not cause adverse skin reactions. This finding is significant as it demonstrates the biocompatibility of these formulations, making them suitable for further development and application in dermatological or cosmetic products. The test results reinforce the potential of these formulations for safe and effective use, aligning with the primary goal of ensuring product safety before clinical or consumer application [17-20].

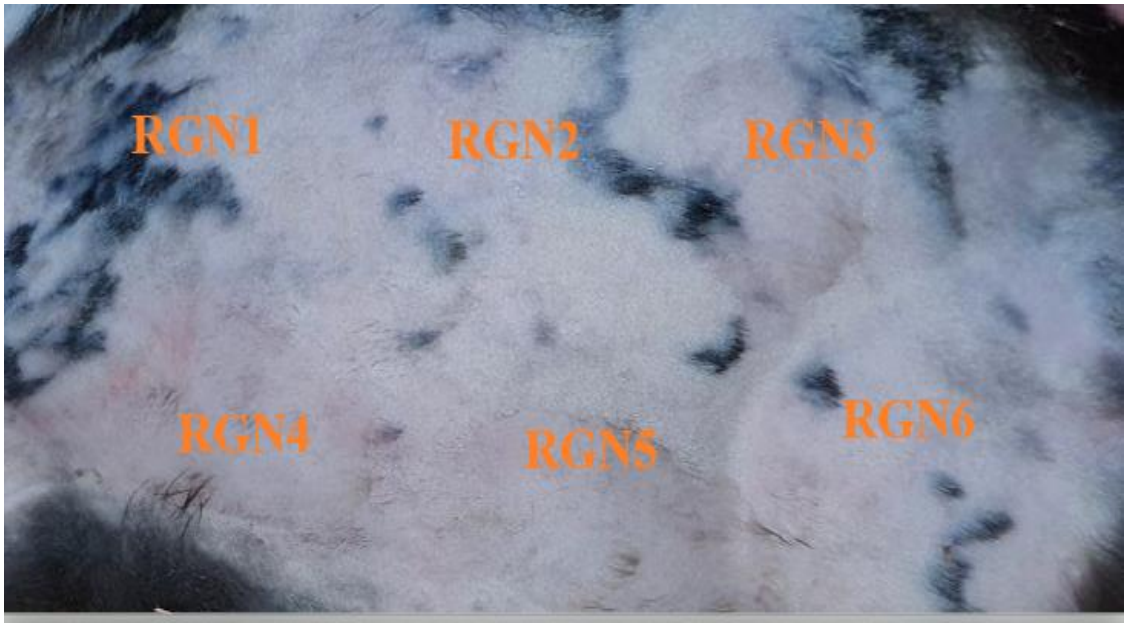

Figure (6): Skin of experimental rabbit that explain there was not skin irritation after application of retinol loaded RGN1– RGN6 formulations.

3.3.6. Biological Membrane Permeation Study Using Retinol as Therapeutic Model

The permeability coefficient (cm/min) was determined by calculating the retinol flux (mg/min) based on experimental data. The results of the ex-vivo skin permeation study indicated that the permeability coefficient of retinol was significantly higher ($p \leq 0.05$) for the retinol-loaded GN1 formulation (RGN1) and significantly lower ($p \leq 0.05$) for the retinol-loaded GN6 formulation (RGN6), as shown in Table (3). The rank order of retinol release from the retinol-loaded formulations (RGN1–RGN6) was as follows: RGN1 > RGN2 > RGN3 > RGN4 > RGN5 > RGN6, as illustrated in Figure (7). The lower permeability coefficient observed for the RGN6 formulation is likely due to the presence of larger nanoparticles, which increased the diffusional pathway and consequently prolonged the time required for permeation. ANOVA results revealed a significant ($p \leq 0.05$) relationship between the biological membrane permeation parameter and the independent variables [17-20]. The ex-vivo permeation study provides valuable insights into the behavior of retinol-loaded formulations across biological membranes. The significantly higher permeability coefficient observed for the RGN1 formulation suggests that it is more effective in facilitating the permeation of retinol, likely due to its optimal nanoparticle size and composition. Conversely, the RGN6 formulation exhibited the lowest permeability coefficient, attributed to its larger nanoparticles, which create a more complex and extended diffusion pathway. This finding underscores the critical role of nanoparticle size in influencing drug delivery efficiency. The ANOVA results further confirm the importance of optimizing formulation parameters to enhance permeation, making this study a key step in the development of effective topical retinol therapies.

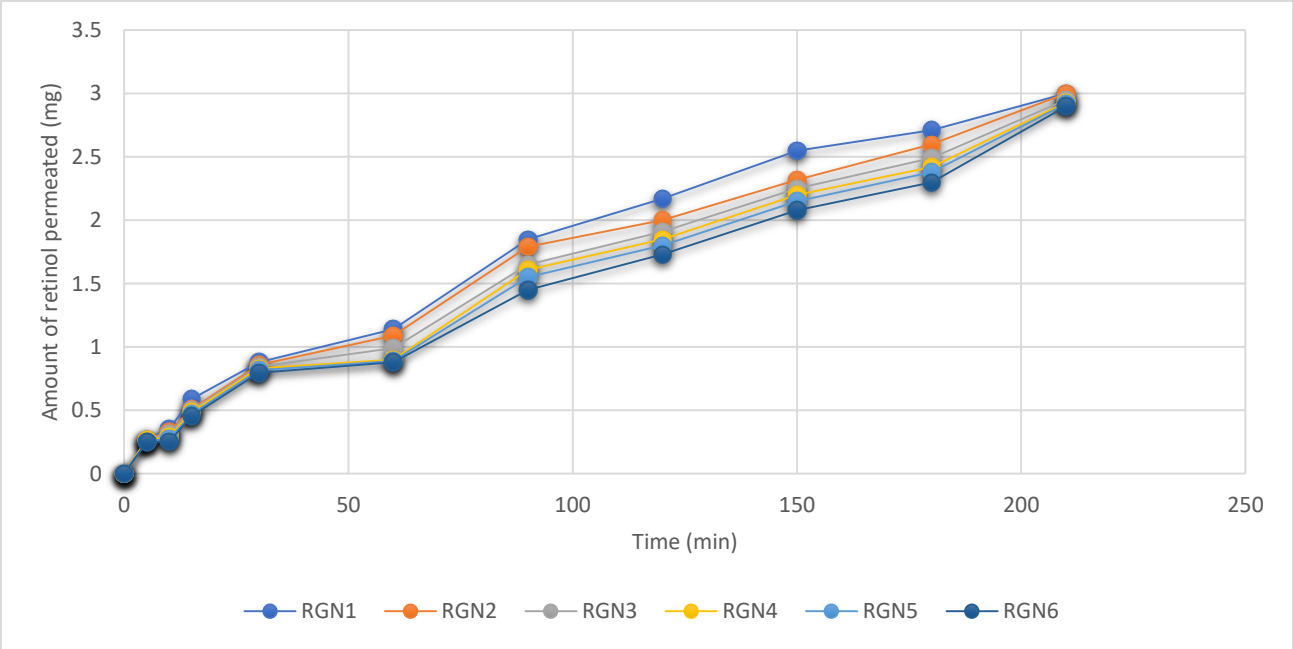

Figure (7): Permeation of retinol loaded RGN1– RGN6 formulations through biological membranes.

Table (3): Slope and permeation coefficient for RGN1-RGN6 formulations through experimental membrane.

Formulation code	Slope (mg/min)	Permeability coefficient (cm/min)
RGN1	0.0141	0.00023949
RGN2	0.0136	0.000230997
RGN3	0.0132	0.000224203
RGN4	0.0130	0.000220886
RGN5	0.0129	0.000219108
RGN6	0.0126	0.000214012

*Values are expressed as mean ± SD (n=3).

4. Conclusion:

The comprehensive research on the green formulation of anti-aging gel masks containing essential oil nanoemulsions (NE1–NE6) has yielded promising results. The thermodynamic stability study and various assessment processes underscore the efficacy and suitability of these eco-friendly nanoemulsions as nanocarriers for delivering various therapeutic agents. The incorporation of natural materials into the preparation of nanoparticles (NE1–NE6) enhances their therapeutic potential, leveraging the beneficial chemical components inherent in these materials. These components play a critical role in neutralizing factors that contribute to aging, thereby mitigating the effects of aging on the body, particularly the skin. The combination of natural therapeutic agents and advanced nanoemulsion technology results in a product that is both environmentally friendly and highly effective in reducing the effects of aging. The success of these formulations paves the way for future exploration and development of natural, sustainable skincare products that cater to the needs of aging skin. This research contributes valuable insights into developing sustainable skincare formulations that harness the power of natural ingredients.

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