Formulation and evaluation of nanoemulsion-based nanocream using green ingredients exhibiting enhanced performance characteristics

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Abstract-The present study focuses on the formulation and evaluation of a nanoemulsion-based nanocream using green ingredients, aimed at enhancing performance, stability, and sustainability. The nanoemulsion was developed through the low-energy phase inversion temperature (PIT) method, which successfully protected green bioactive compounds like vitamin E, cinnamon oil, jojoba oil, and peppermint oil from degradation. A series of nanoemulsions were prepared using varying ratios of oils and surfactants and evaluated for thermodynamic stability, transparency, and droplet size. The optimized nanoemulsion, with a mean droplet size of 121.3±1.19 nm and a low polydispersity index (PDI) of 0.094±0.001, demonstrated high uniformity and stability. This optimized nanoemulsion was further used as the cream's aqueous phase, forming a nanocream that exhibits enhanced permeation of nanoscale bioactives through a membrane and improved overall performance characteristics. *In vitro* membrane permeation studies revealed that the optimized nanocream achieved a permeation rate of 97.15%, substantially outperforming the control cream. *In vitro* antimicrobial studies showed comparable efficacy to standard market preparations containing synthetic agents. The nanocream also demonstrated long-term stability over six months, maintaining structural integrity without phase separation or significant changes in pH and spreadability. The nanoemulsion-based nanocream formulated with eco-friendly ingredients hence offers enhanced skin permeation, superior bioactive delivery, and stable performance, making it a promising candidate for topical skincare and antimicrobial applications.

Keywords: Nanoemulsion, Nanocream, Green ingredients, Phase inversion temperature, Bioactive oils, Skin permeation

INTRODUCTION

Nanoemulsions consist of oil droplets dispersed in water, stabilized by surfactants, with droplet sizes typically ranging from 20 to 200nm—much smaller than those in conventional emulsions. These nano-sized droplets enhance the delivery, and bioavailability of water-insoluble drugs and bioactive compounds, making nanoemulsion-based systems highly effective for drug formulations and cosmetics [1]. By reducing droplet size, nanoemulsions improve skin penetration, enabling controlled release of bioactives and providing better therapeutic outcomes for topical applications. Additionally, they offer improved stability for sensitive ingredients like vitamins and essential oils, making them ideal for treating skin conditions without causing irritation or allergic reactions often seen with synthetic compounds [2, 3].

Vitamin E (tocopherol) is well-known for its skin-preserving properties, protecting the skin from oxidative stress caused by environmental factors like UV radiation and pollution. It also plays a crucial role in maintaining healthy skin by promoting hydration and aiding wound healing, making it essential in formulations designed to treat dry and damaged skin [4]. Cinnamon oil, derived from the bark of cinnamon trees, is recognized for its anti-inflammatory and antimicrobial properties. It is particularly effective against acne-causing bacteria and helps reduce redness and irritation, making it an excellent ingredient for treating various inflammatory skin conditions [5]. Jojoba oil, a well-known emollient, closely mimics the skin's natural oils, helping to maintain hydration without clogging pores, making it suitable for acne-prone skin. It also has anti-inflammatory properties that soothe irritated skin. Peppermint oil is widely used for its cooling and soothing effects, helping to alleviate skin irritation and itching. Additionally, it has antimicrobial properties and can be used to treat skin infections [6]. Natural waxes like beeswax and cocoa butter are excellent emollients that form a protective barrier on the skin's surface, helping to prevent moisture loss and promote the healing of dry, cracked skin. Their anti-inflammatory and antibacterial properties also help soothe and protect the skin from external irritants [7].

The development of nanoemulsion-based creams using green technology represents a major advancement in dermatological formulations [8]. Green technology emphasizes the use of environmentally friendly processes and ingredients, ensuring that the final product is both sustainable and safe for long-term use [9, 10]. The nanoemulsion-based nanocream in this study offers several advantages over traditional skincare products, notably enhancing the bioavailability of poorly soluble bioactive compounds, which ensures effective skin absorption and therapeutic efficacy [11, 12].

Low-energy emulsification techniques, such as the phase inversion temperature (PIT) method, are used to develop nanoemulsions. PIT method and other low-energy methods for nanoemulsions are advantageous, compared to high-energy techniques, especially those containing heat-sensitive bioactives. High-energy methods utilize mechanical devices to disrupt oil phases, generating heat and risking the degradation of bioactives. In contrast, low-energy methods rely on the system’s internal

chemical energy, requiring minimal energy input and less expensive equipment [13]. This leads to lower preparation costs, better sustainability, and reduced risk of damage to sensitive compounds like proteins and peptides [14]**.** Additionally, low-energy methods avoid the issues of poor productivity and heat-induced component deterioration associated with high-energy techniques, making them more suitable for pharmaceutical and food applications [15-16]**.**

The PIT method is a widely used technique for developing nanoemulsions by inducing phase inversion through temperature changes. In this method, polyethoxylated surfactants, such as Tween 80, which are hydrophilic at lower temperatures, typically form oil-in-water (O/W) emulsions. However, when the system is heated, the polyoxyethylene groups in these surfactants undergo dehydration, making the surfactants more lipophilic. As a result, the spontaneous curvature of the surfactant molecules shifts, leading to the formation of a water-in-oil (W/O) emulsion. The temperature at which this phase inversion occurs is referred to as the phase inversion temperature (PIT), also known as the HLB (Hydrophilic-lipophilic balance) temperature. To produce a nanoemulsion using the PIT method, the O/W emulsion system is heated until the phase inversion occurs. Subsequently, the system is rapidly cooled with continuous stirring, a process called temperature quenching. This cooling reverses the phase inversion, transforming the system back to an O/W emulsion. The turbulence caused by the rapid temperature change promotes the formation of nano-sized oil droplets, resulting in a stable O/W nanoemulsion [17-19].

Overall, the objective of this research paper is to develop and evaluate a nanoemulsion-based nanocream formulated with green ingredients, including vitamin E, cinnamon oil, jojoba oil, and peppermint oil. Using a sustainable and innovative preparation method, this study seeks to harness the benefits of nanoemulsion technology combined with a cream base to create a nanocream that enhances overall performance characteristics and antimicrobial efficacy.

EXPERIMENTAL

Materials

All ingredients are of natural origin and sustainable. Vitamin E (RejuveNaturals USA), cinnamon oil (Now Foods USA), peppermint oil (Now Foods USA), jojoba oil (US Organic), Tween 80 (Traverse Bay Bath and Body), glyceryl monostearate (MYOC), cetostearyl alcohol (MYOC), glycerine (H&B Oils Center Co.), beeswax (YASNAY), cocoa butter (SaaQin) and distilled water. All other chemicals and reagents used in this study were of the highest purity and of analytical grade.

Methods

Development of UV-spectrophotometric method for estimation of bioactive oil. A UV-spectrophotometric method for vitamin E (Vit E) was developed to estimate the amount of Vit E permeating through the membrane during in vitro membrane permeation studies of the nanocream. To achieve this, a calibration curve of Vit E oil in methanol was generated using a UV spectrophotometer, as described in a previous study [20]. A stock solution of Vit E was prepared in methanol, and five solutions of varying concentrations (10, 25, 50, 75, and 100 µg/mL) were obtained by appropriate dilution of the stock solution. The absorbance of each solution was measured at 290 nm in triplicate. The calibration curve was plotted by graphing absorbance against concentration. The correlation coefficient and regression equation were determined through regression analysis.

Determination of the phase inversion temperature by turbidity method. When an emulsion system is heated to its phase inversion temperature (PIT), its appearance changes and becomes turbid. This occurs because, at elevated temperatures, non-ionic surfactants like Tween 80 undergo dehydration, causing the oil-in-water emulsion to invert into a water-in-oil emulsion. Therefore, the temperature at which this visual change occurs is identified as PIT [21-22]. Thus, the PIT of the system was determined by visual observation of turbidity during heating. To begin, a nanoemulsion was prepared via water titration, where 5% of an oil mixture (comprising vitamin E, cinnamon oil, jojoba oil, and peppermint oil in equal proportions) and 25% of a surfactant mixture (Tween 80 and glycerine in a 3:1 ratio) were combined. Then, 70% distilled water was slowly added to this mixture with continuous stirring, leading to the formation of a transparent nanoemulsion due to the low oil and high surfactant concentration. The prepared system was then heated while stirring, and the temperature at which the appearance changed (turbidity or cloudiness) was observed and PIT was noted. This procedure was repeated three times to ensure the accuracy of the PIT measurement.

Development of nanoemulsion system by PIT method. The formulation of the nanoemulsion was carried out using a PIT method to develop an optimal system. The oil phase used consisted of a blend of 30% vitamin E, 30% cinnamon oil, 20% jojoba oil, and 20% peppermint oil, while Tween 80 was chosen as the primary surfactant. Various ratios of the oil phase and surfactant were tested to achieve the best nanoemulsion, with the details provided in the Table 1. For the preparation of 10 grams of the nanoemulsion, the combined oil phase and surfactant were weighed to 1.2 grams (12% of the total formulation). Additionally, 3.5% glycerine (0.35 grams), 2% cetostearyl alcohol (0.2 grams), and 2.5% glyceryl monostearate (0.25 grams) were included as co-surfactants to further stabilize the nanoemulsion.

To begin the formulation, the oil phase was mixed with the surfactant and co-surfactants in a glass vial and heated to a temperature 10°C above the PIT. In a separate vial, 80% of the total distilled water (8

grams) was also heated to 10°C above the PIT. Once both phases were preheated, the water phase was added to the heated oil and surfactant mixture while stirring continuously at 300 rpm, with the system maintained at the elevated temperature. Stirring was continued for 10 minutes, during which the system appeared turbid and inhomogeneous. Following this, the entire system was rapidly cooled to 25°C by placing the glass vial in ice-cold water, inducing temperature quenching. Stirring was maintained during this cooling process to ensure proper emulsification, leading to the formation of a transparent and stable nanoemulsion. A total of 9 nanoemulsion systems with varying ratios of oil phase and surfactant were prepared.

Evaluation of Developed Nanoemulsion System

Thermodynamic stability testing. The thermodynamic stability of the nanoemulsion formulations was evaluated through centrifugation and heating-cooling (H/C) cycle tests. In the centrifugation test, each formulation was subjected to a centrifuge at 3500 rpm for 15 minutes. After centrifugation, the formulations were visually inspected for any signs of instability, such as phase separation or creaming, which would indicate poor stability.

For the heating-cooling cycle test, the formulations were alternately exposed to temperatures of 4°C and 45°C, with each cycle lasting 24 hours at each temperature. After completing the cycles, the formulations were examined for any changes in stability, including phase separation, turbidity, or other signs of instability. These tests were conducted to ensure the nanoemulsions maintained their structural integrity under varying conditions.

Determination of transparency of the system by percentage transmittance measurement. The transparency of the nanoemulsion formulations was assessed by determining the percentage of transmittance (% transmittance), which provides an indication of droplet size within the nanometer range. To measure % transmittance, all formulations were first diluted 50 times with distilled water to ensure accurate readings. The diluted samples were then analyzed using a UV spectrophotometer set at a wavelength of 650 nm, with distilled water used as a blank reference.

Mean droplet size and polydispersity index (PDI) determination of optimum nanoemulsion system. The mean droplet size and polydispersity index (PDI) of the optimum nanoemulsion system was determined using the dynamic light scattering (DLS) technique with a Zetasizer (Malvern Instruments). To prepare the sample for measurement, the nanoemulsion formulation was diluted 20 times (1:20) with distilled water to ensure accurate detection of droplet size distribution. The diluted sample was then analyzed at a scattering angle of 90° and a temperature of 25°C using the Zetasizer. This method provided detailed information on the average droplet size and the uniformity of the droplet size distribution (PDI), which are critical parameters for assessing the stability and performance of the nanoemulsion.

Development of Nanoemulsion-based Nanocream.

The nanocream was developed using a novel approach, incorporating nanoemulsion as the aqueous phase in the cream formulation as shown in Fig. 1. Nanoemulsion, with its water-like consistency, was used in place of water, improving the stability of nanosized droplets and reducing the risk of coalescence of bioactive oils. This is due to the highly viscous and thick nature of the cream's oily base, which restricts the movement of nanoemulsion droplets, preventing them from merging [23-25].

For the oil phase of the nanocream, beeswax and cocoa butter were utilized as the cream's oily base, while cetostearyl alcohol and glyceryl monostearate served as emulsifiers. The aqueous phase consisted of the developed optimum nanoemulsion, containing vitamin E, cinnamon oil, jojoba oil, and peppermint oil, along with glycerine as a humectant. Different ratios of the oil and aqueous phases were used to prepare six nanocream formulations, as shown in the Table 2.

To begin the nanocream formulation, the ingredients for the oil phase—beeswax, cocoa butter, glyceryl monostearate, cetostearyl alcohol—were weighed, added to a beaker, and melted while stirring at 65°C. Simultaneously, in a separate beaker, the aqueous phase, comprising the developed nanoemulsion and glycerine, was heated to 55°C, a temperature well below the PIT of the nanoemulsion. The preheated aqueous phase was then slowly added to the preheated oil phase with continuous stirring while gradually cooling the mixture to room temperature. The cooling process was conducted slowly to ensure a smooth emulsion. After the complete addition of the aqueous phase to the oil phase, stirring and cooling were continued until a homogeneous and viscous nanocream was formed. This will lead to the development of an oil-in-water nanocream.

Impact of nanoemulsion utilization as the aqueous phase in cream formulation. To examine the effect of using nanoemulsion as the aqueous phase in cream formulation, two additional cream formulations were prepared for comparison. These formulations were designed to assess the role of nanoemulsion in enhancing the stability and performance of the cream.

In the first formulation (C1), water was used in place of nanoemulsion as the aqueous phase, while the oil phase composition remained identical to the optimum nanocream formulation among the six previously developed nanocreams. This allowed for a direct comparison of the effect of replacing the nanoemulsion with water in the cream formulation. Similarly in the second formulation (C2), again water was used as the aqueous phase; however, to compensate for the absence of the surfactant Tween 80, which was present in the nanoemulsion, Tween 80 was added to the oil phase. Since Tween 80 is an excellent emulsifier, its inclusion ensured that the emulsifying properties of the formulation were maintained even without the nanoemulsion. Both formulations were prepared using the same method as previously described.

Evaluation of Developed Nanocream

Physical appearance and pH of nanocream. The organoleptic characteristics of all nanocream formulations were evaluated through visual observation and tactile assessment. The color, texture, homogeneity, and any signs of phase separation were examined by inspecting the appearance of each formulation. To further assess homogeneity and texture, a small amount of nanocream was pressed and rubbed between the thumb and index finger. This allowed for the evaluation of immediate skin feel, including sensations of stiffness, grittiness, greasiness, and overall smoothness. For the pH determination, one gram of each nanocream formulation was dispersed in 25 mL of distilled water. The pH of the resulting dispersion was measured using a calibrated pH meter to ensure the formulations were suitable for topical application and within the desired pH range for skin compatibility.

Stability determination by centrifugation test. To evaluate the stability of the oil-in-water (O/W) nanocream formulation against creaming, a centrifugation test was conducted. Creaming is a common instability reaction in O/W emulsions, where the dispersed oil phase may separate and rise to the top, forming a layer of oil droplets. This test helps to confirm that the developed nanocream is stable, well-emulsified, and homogenous [26].

For the centrifugation test, 5 grams of nanocream were placed in a centrifuge tube and centrifuged at 3500 rpm for 20 minutes. After centrifugation, the nanocream was carefully inspected for any signs of creaming, phase separation, or visible layers of oil droplets. The absence of these signs would indicate that the nanocream has good stability and resistance to creaming under accelerated conditions.

Spreadability determination. To determine the spreadability of the nanocream, a sample weighing 1 gram was placed at the center of a square glass plate (dimensions: 10 cm × 20 cm). A second, identical glass plate was carefully placed on top of the nanocream, and a 1.5 kg weight was applied at the center for 1 minute to allow the nanocream to spread between the plates. After the designated time, the plates were separated, and the spread diameter of the nanocream was measured in four different directions: vertical, horizontal, and two diagonals. The average of these four measurements was calculated to obtain the final Spreadability reading, ensuring an accurate representation of the nanocream's spreading capacity. The spreadability of the nanocream was compared to that of a commercial moisturizing cream, Monange® (manufactured by Savoy Industria de Cosmeticos, Goiania, Brazil).

***In vitro* membrane permeation studies of nanocream.** For the *in vitro* membrane permeation study, the optimum nanocream formulation was evaluated and compared with the normal cream formulation (C2), where no nanoemulsion was used as the aqueous phase. The permeation study employed the dialysis bag method [27]**.** A dialysis membrane (Dialysis membrane-150; LA401-5MT; average flat width 42.44 mm and average diameter 25.4 mm, HiMedia Laboratories, Mumbai, India) was activated

by immersing it in distilled water for 24 hours. After activation, one end of the dialysis membrane was securely tied with a thread. Five grams of the nanocream formulation were then loaded into the dialysis membrane through the other end, which was subsequently tied to ensure no leakage occurred. The sealed dialysis bag, containing the nanocream, was submerged in 500 mL of distilled water at a controlled temperature of 37°C. The system was stirred continuously at 200 rpm. At specified time intervals (30 min, 1 h, 2 h, 3 h, etc.), 5 mL samples were withdrawn from the beaker. After each withdrawal, an equivalent volume of distilled water was added to maintain sink conditions. The collected samples were filtered, diluted with methanol, and analyzed for the permeation of vitamin E using a UV spectrometer at 290 nm, calibrated against a standard curve of vitamin E in methanol. In parallel, the permeation study was conducted on the normal cream (C2) using the same procedure, and the results were compared with those of the optimum nanocream.

Determination of *in vitro* antimicrobial activity of nanocream. To evaluate antibacterial activity using the well diffusion assay, Nutrient Agar (28 g) was dissolved in 1 litre of distilled water and sterilized by autoclaving at 121°C under 15 lbs pressure for 15 minutes. The sterilized media was poured into plates and allowed to solidify under laminar airflow. A bacterial suspension of E. coli and S. aureus (10^8 CFU/mL) was prepared and standardized. A 100 µl aliquot of the bacterial inoculum was spread evenly across the surface of each solidified agar plate using a sterile spreader. Two wells, each 6 mm in diameter, were created in the agar using a sterile cork-borer. One well was filled with the optimum nanocream, and the other with a standard ointment (Veldine-OZ containing 5% w/w povidone-iodine and 1% w/w ornidazole ointment manufactured by Valentis Pharma). Plates were left at room temperature for 30 minutes to allow diffusion and then incubated at 37°C for 18-24 hours. After incubation, the zones of inhibition (ZOI) were measured in millimetres, including the diameter of the well, using a ruler placed on the inverted Petri dish against a black background. The clear zones around the wells indicated the antimicrobial activity of the tested compounds. Minimum inhibitory concentration (MIC) was determined using the broth dilution method with two-fold serial dilutions in nutrient broth medium, following procedures described in previous studies [28-29].

Evaluation of nanocream surface charge via zeta potential analysis. To evaluate the surface charge of the nanocream, 1 gram of the optimum nanocream sample was diluted 200-fold by dispersing it into 200 mL of distilled water. The mixture was then shaken thoroughly and stirred to ensure homogeneous dispersion of the nanodroplets. After thorough mixing, the dispersion was filtered to remove any large particles from the cream base. The surface charge of the nanodroplets was then determined by measuring the zeta potential using a Zetasizer (Malvern Instruments). This measurement provides insight into the electrostatic stability and surface characteristics of the nanocream.

Long term stability testing of nanocream. To evaluate the long-term stability of the optimum nanocream formulation, the product was subjected to a storage stability study. The formulated nanocream was carefully packed in a glass beaker and stored at room temperature (25 ± 2°C) for a period of six months. Over this duration, any potential instability mechanisms were monitored, including changes in physical appearance, spreadability, pH, and phase separation. After the six-month storage period, the nanocream was reevaluated for these parameters. Physical appearance was visually inspected to note any alterations in texture, color, or homogeneity. Phase separation was assessed by observing any distinct layers that may have formed, indicating instability. The pH of the formulation was measured by dispersing a small sample in deionized water and using a calibrated pH meter. Lastly, spreadability was determined using a glass plate method to quantify any changes in the ease of application compared to its initial state. These tests provided valuable insights into the formulation's ability to retain its original properties over time, ensuring its long-term stability and effectiveness

RESULT AND DISCUSSION

Development of UV- spectrophotometric method for estimation of bioactive oil. In calibration curve of Vit E in methanol, linearity ranges from 10 to 100 µg/mL in methanol with a correlation coefficient (r) of 0.996. The regression equation was found to be Y=0.0062x + 0.1995. (Fig. 2).

Evaluation and selection of optimum nanoemulsion formulation. A total of nine nanoemulsion systems were developed by varying the ratios of the oil phase and surfactant. These formulations were evaluated based on their thermodynamic stability and transparency to select the optimum nanoemulsion system for further use in nanocream formulation. The results of the thermodynamic stability tests and the transparency evaluation are presented in the Table 3. Among the nine formulations, NE9, NE8, NE7, and NE6 exhibited a transparent to translucent appearance, indicative of nanosized droplets [30]**.** NE6 was selected as the optimum nanoemulsion formulation due to its superior composition, which included a higher proportion of bioactive oils and a lower surfactant concentration compared to NE9, NE8, and NE7. Additionally, NE6 demonstrated thermodynamic stability as well.

Further the evaluation of NE6 revealed a mean droplet size of 121.3±1.19 nm (Mean ± SD; n=3) and a PDI of 0.094±0.001 (Mean ± SD; n=3). The nano size droplet confirms its classification as a nanoemulsion, while the relatively low PDI value reflects a high degree of monodispersity, indicating uniform droplet size distribution. This uniformity is a desirable characteristic for the nanoemulsion system [31-32]**.**

Thus, NE6 nanoemulsion formulation was chosen as the optimum nanoemulsion and further used for the formulation of nanocream.

Evaluation and selection of optimum nanocream formulation. The results of the evaluation of all six nanocream formulations are presented in the Table 4. Each formulation exhibited good emulsification and homogeneity, with no signs of grittiness. The successful emulsification was further supported by the centrifugation test, in which all formulations demonstrated stability, with no phase separation. The pH values of all nanocreams ranged within the normal skin pH range, suggesting minimal risk of skin irritation upon application. It was observed that formulations with an oil phase exceeding 30% and an aqueous phase below 65% were thick and hard in texture. Additionally, a slight increase in pH was noted as the total oil phase increased.

The spreadability of the nanocreams was compared to that of a commercial moisturizing cream (Monange® by Savoy Industria de Cosmeticos, Goiania, Brazil). Among all formulations, NC4 had a spreadability value closest to the commercial cream, indicating that NC4 has optimal spreadability, which would enhance ease of application on the skin. Based on this evaluation, NC4 was selected as the optimum nanocream formulation. In NC4, the total nanoemulsion used as the aqueous phase was 66%, allowing for the incorporation of significant quantities of bioactive oils: 891 mg of vitamin E, 891 mg of cinnamon oil, 594 mg of jojoba oil, and 594 mg of peppermint oil as nanosized droplets (in 66 g of total nanoemulsion as aqueous phase) in 100 grams of nanocream.

Effect of using nanoemulsion as aqueous phase in nanocream formulation. To evaluate the impact of incorporating nanoemulsion as the aqueous phase in the nanocream formulation, two additional cream formulations C1 and C2 were prepared using the composition of the optimum nanocream (NC4), as detailed in the Table 5. The evaluation results are presented in the Table 6. When water alone was used as the aqueous phase in C1, the formulation failed to emulsify, resulting in an unstable cream that could not form properly. This indicates that cetostearyl alcohol and glyceryl monostearate alone were insufficient to emulsify 70% of the aqueous phase in the cream. However, when 5% Tween 80 was added along with water in the C2 formulation, the cream emulsified successfully, though it lacked homogeneity and displayed small, soft lumps compared to the smoother NC4 formulation. This demonstrates that the presence of Tween 80 in the nanoemulsion not only reduced the size of bioactive oil droplets to the nanoscale but also facilitated the emulsification of two phases of cream, contributing to a smoother and more homogenous cream. Thus, the use of nanoemulsion as the aqueous phase, with Tween 80 playing a dual role, significantly improves the texture, emulsification, and overall quality of the nanocream formulation.

Overall, the NC4 nanocream formulation was selected as the optimum formulation based on its superior emulsification, homogeneity, and spreadability. It was further evaluated for *in vitro* membrane permeation studies, *in vitro* antimicrobial activity long term stability and other characteristics, to confirm its enhanced performance and potential for therapeutic effectiveness.

***In vitro* membrane permeation studies of nanocream.** The results of the in vitro membrane permeation study comparing the optimum nanocream formulation (NC4) with the normal cream formulation (C2) are illustrated in the Fig. 3. It was observed that the permeation of bioactive oil Vit E through the dialysis membrane was significantly higher for the nanocream (NC4) than for the normal cream (C2). At the end of the 10-hour study, the NC4 nanocream demonstrated a permeation rate of 97.15 ± 2.33% (Mean ± SD; n=3), whereas the C2 cream showed only 62.46 ± 2.18% (Mean ± SD; n=3) permeation. This substantial difference in permeation between the two formulations can be attributed to the nanosized droplets in the NC4 nanocream, which allow for more efficient penetration through the membrane. These findings suggest that the nanoscale structure of the nanocream significantly improves its delivery potential, pointing to enhanced therapeutic effectiveness in comparison to conventional cream formulations. The superior in vitro permeation performance of the NC4 nanocream indicates its potential for better skin absorption and efficacy in practical applications.

***In vitro* antimicrobial activity and minimum inhibitory concentration (MIC) of nanocream.** The antibacterial activity of the optimum nanocream formulation (NC4) and the standard ointment was assessed using the well diffusion assay (Fig.4). The results showed that the nanocream exhibited significant antibacterial effects against both *E. coli* and *S. aureus*. The zones of inhibition were measured at 16.33±4.16 mm for *E. coli* and 29.33±4.73 mm for *S. aureus*, indicating that the formulation had strong antibacterial properties (Table 7). To further investigate its efficacy, the minimum inhibitory concentration (MIC) test was performed to determine the lowest concentration at which the formulation could inhibit bacterial growth. For *E. coli*, a gram-negative bacterium, the MIC of the nanocream formulation was determined to be 62.5 µg/mL, while for *S. aureus*, a gram-positive bacterium commonly associated with skin infections, the MIC was found to be lower at 31.25 µg/mL (Table 8). The study concluded that the antimicrobial efficacy of the developed nanocream was comparable to that of the standard market preparations containing synthetic agents, making it a promising candidate for treating bacterial skin infections.

The observed superior antibacterial activity of the developed nanocream against *S. aureus* compared to *E. coli* can be attributed to the simpler cell wall structure of *S. aureus*, which contains a thick peptidoglycan layer. This structural feature makes it easier for the bioactive compounds in the nanocream to interact with and penetrate the cell wall of *S. aureus*. In contrast, *E. coli* has a more complex outer membrane with lipopolysaccharides, which may act as a barrier, reducing the accessibility of bioactive ingredients [33]. Nevertheless, the nanocream still demonstrated significant antibacterial activity against *E. coli*, indicating its potential for broad-spectrum antimicrobial applications.

The development of a nanoemulsion-based nanocream has enhanced the efficacy of green bioactive ingredients. The smaller droplet size of the nanocream increases the surface area available for interaction with bacterial cells, thereby improving the absorption and penetration of bioactive compounds into

deeper layers of the skin [34]. This is especially beneficial for targeting skin pathogens [35]. Furthermore, the use of green technology in the formulation offers a safer and environmentally friendly alternative to synthetic antimicrobial agents, meeting the growing consumer demand for natural and sustainable skincare solutions. Overall, the nanocream formulation has shown promising broad-spectrum antibacterial activity against both *S. aureus* and *E. coli*.

Nanocream surface charge and impact on skin barrier function: The zeta potential of the optimum nanocream (NC4) was measured at -23 mV, indicating that the nanodroplets in the formulation possess a negative surface charge. This negative charge is beneficial for enhancing the skin's barrier function. Negatively charged particles can strengthen the skin's physical barrier by forming an occlusive layer on the surface of the stratum corneum, which reduces trans epidermal water loss (TEWL) and aids in skin moisturizing [36]. Additionally, the negative charge of the particles supports the recovery of a disrupted skin barrier, further promoting skin repair [37]. These findings suggest that the developed nanocream not only aids in moisturizing but also plays a role in repairing and protecting the skin barrier.

Long term stability testing of nanocream. The long-term stability of the selected optimum nanocream formulation NC4 was assessed after storing it at room temperature (25 ± 2°C) for a period of six months. The results of the stability study, summarized in Table 9, demonstrated that the formulation maintained its stability throughout the six-month duration. Notably, there were no signs of phase separation, and the nanocream exhibited a homogeneous appearance. Furthermore, measurements indicated that there were no significant changes in pH, suggesting that the formulation's integrity was preserved. Additionally, the spreadability of the nanocream remained consistent with the initial measurements. These findings collectively indicate that the novel nanoemulsion-based nanocream is stable for long-term storage, affirming its potential for practical applications in skincare.

CONCLUSIONS

In this study, a nanoemulsion-based nanocream was successfully formulated using natural and sustainable ingredients. An optimum nanoemulsion with nanosized droplets of bioactive oils and high uniformity was first developed. This nanoemulsion was then used as the aqueous phase in the nanocream formulation. The resulting nanocream demonstrated enhanced bioactive permeability, superior spreadability, homogeneity, consistency, comparable antimicrobial activity with synthetic agents, indication of enhanced skin barrier function and long-term stability. The use of the low-energy phase inversion temperature method for nanoemulsion preparation preserved the green ingredients, contributing to the overall efficacy of the formulation, while reducing preparation costs. This novel approach of incorporating nanoemulsion as the aqueous phase in cream formulations shows promise for

developing stable nanocream with improved performance characteristics, paving the way for future applications in skin care, cosmetic, and dermatological products.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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TABLE

Table 1. Formulation batches of nanoemulsion

NE code	Oil phase (gm)	Tween 80 (gm)	3.5% Glycerine (gm)	2.5% GMS (gm)	2% CSA (gm)	80% Water (8 gm)	Total (gm)
NE1	0.72	0.48	0.35	0.25	0.2	8	10
NE2	0.66	0.54	0.35	0.25	0.2	8	10
NE3	0.6	0.6	0.35	0.25	0.2	8	10
NE4	0.54	0.66	0.35	0.25	0.2	8	10
NE5	0.48	0.72	0.35	0.25	0.2	8	10
NE6	0.45	0.75	0.35	0.25	0.2	8	10
NE7	0.42	0.78	0.35	0.25	0.2	8	10
NE8	0.39	0.81	0.35	0.25	0.2	8	10
NE9	0.36	0.84	0.35	0.25	0.2	8	10

NE, Nanoemulsion; CSA, Cetostearyl Alcohol; GMS, Glyceryl Monostearate

Table 2. Formulation batches of nanocream using nanoemulsion as aqueous phase

NC Code no	Oil phase (%)					Aqueous phase (%)		
	GMS (%)	CSA (%)	Cocoa Butter (%)	Bees Wax (%)	Total Oil Phase (%)	Glycerine (%)	NE (%)	Total Aqueous Phase (%)
NC1	9	9	8	14	40	4	56	60
NC2	8.5	8.5	7	13.5	37.5	4	58.5	62.5
NC3	8	8	6	13	35	4	61	65
NC4	7	7	4	12	30	4	66	70
NC5	6	6	2	11	25	4	71	75
NC6	5.5	5.5	1	10.5	22.5	4	73.5	77.5

NC, Nanocream; NE, Nanoemulsion; CSA, Cetostearyl Alcohol; GMS, Glyceryl Monostearate

Table 3. Result of evaluation of nanoemulsion formulation

NE code	Appearance	(%) Transmittance (Mean ± SD)	Thermodynamic stability testing	
			H/C cycle tests	Centrifugation test
NE1	Turbid	4.67 ± 1.22	Phase separation	Phase separation
NE2	Turbid	3.75 ± 1.89	Phase separation	Phase separation
NE3	Turbid	7.71 ± 2.05	Phase separation	No phase separation
NE4	Turbid	20.26 ± 2.88	Stable	Stable
NE5	Turbid	24.15 ± 2.91	Stable	Stable
NE6	Translucent-transparent	84.74 ± 1.88	Stable	Stable
NE7	Translucent-transparent	92.15 ± 1.05	Stable	Stable
NE8	Transparent	98.17 ± 0.93	Stable	Stable
NE9	Transparent	98.03 ± 0.76	Stable	Stable

n=3; SD, standard deviation; NE, Nanoemulsion; H/C cycle, heating cooling cycle.

Table 4. Result of evaluation of nanocream formulation

NC code no	Appearance	pH (Mean ± SD);	Centrifugation test	Spreadability (mm)
NC1	Dense, thick creams with a firm consistency	6.05± 0.12	Stable	40.5
NC2	Slight dense, thick creams with a firm consistency	5.95 ± 0.09	Stable	46.25
NC3	Slight dense, thick creams with a firm consistency	5.98 ± 0.06	Stable	54.75
NC4	Light, smooth creams, and soft consistency	5.56 ± 0.15	Stable	65.5
NC5	Light, smooth creams, and soft consistency	5.35 ± 0.20	Stable	72.5
NC6	Creamy, fluid-like consistency, more liquid in nature	5.31 ± 0.06	Stable	88.25
Commercial Cream	Monange® - Savoy Industria de Cosmeticos, Goiania, Brazil (Moisturizing cream)			62 ± 0.75

n=3; SD, standard deviation; NC, Nanocream;

Table 5. Normal cream formulation by using water as aqueous phase

Cream Code no	Oil phase (%)							Aqueous phase (%)		
	GMS (%)	CSA (%)	Cocoa butter (%)	Bees wax (%)	Bioactive oil blend* (%)	Tween 80 (%)	Total oil phase (%)	Gly (%)	Water (%)	Total aqueous phase (%)
C1	7	7	4	12	2.97	-	32.97	4	63.03	67.03
C2	7	7	4	12	2.97	5	37.97	4	58.03	62.03

CSA, Cetostearyl Alcohol; GMS, Glyceryl Monostearate; Gly, Glycerine; *Bioactive oil blend comprises vitamin E, cinnamon oil, jojoba oil, and peppermint oil in a 3:3:2:2 ratio

Table 6. Result of evaluation of normal cream formulation

Cream code no	Appearance	pH (Mean ± SD); n=3	Centrifugation test	Spreadability (mm)
C1	Not emulsified, unstable	-	-	-
C2	Light cream soft consistency with lumps, not homogenous	5.06 ± 0.16	Stable	75.25

n=3; SD, standard deviation

Table 7 Antibacterial activity of nanocream against *E. coli and S. aureus* by well diffusion method

Sample	Zone of Inhibition in (mm) Mean±SD	
	E. coli	***S. aureus***
Nanocream	16.333±4.163	29.333±4.725
Std. Ointment (Povidone Iodine + Ornidazole)	30.666±2.516	30.666±1.154

n=3; SD, standard deviation

Table 8. Minimum inhibitory concentration of nanocream against *E. coli and S. aureus* by broth dilution method

Sample	Strain	Minimum Inhibitory Concentration (MIC) (µg/mL)
Nanocream	*E. coli*	62.50 µg/mL
	S. aureus	31.25 µg/mL

Table 9. Long-term stability testing of optimum nanocream formulation NC4

Time	Appearance	pH (Mean ± SD)	Centrifugation test	Spreadability (mm)
Initial	Light, smooth creams, and soft consistency	5.56 ± 0.15	Stable	65.5
After 6 months	Light, smooth creams, and soft consistency	5.23 ± 0.26	Stable	62.25

n=3; SD: standard deviation.

FIGURE CAPTION

Fig. 1. Diagrammatic representation of nanoemulsion-based nanocream formulation

Fig. 2. Calibration curve of vitamin E in methanol

Fig. 3. In vitro membrane permeation of nanocream (NC4) and normal cream (C2).

Fig. 4. Zone of inhibition shown by nanocream (NC) and standard ointment (STD) against *E.coli* (P1, P2 and P3) and *S. aureus* (P4, P5 and P6).

FIGURE

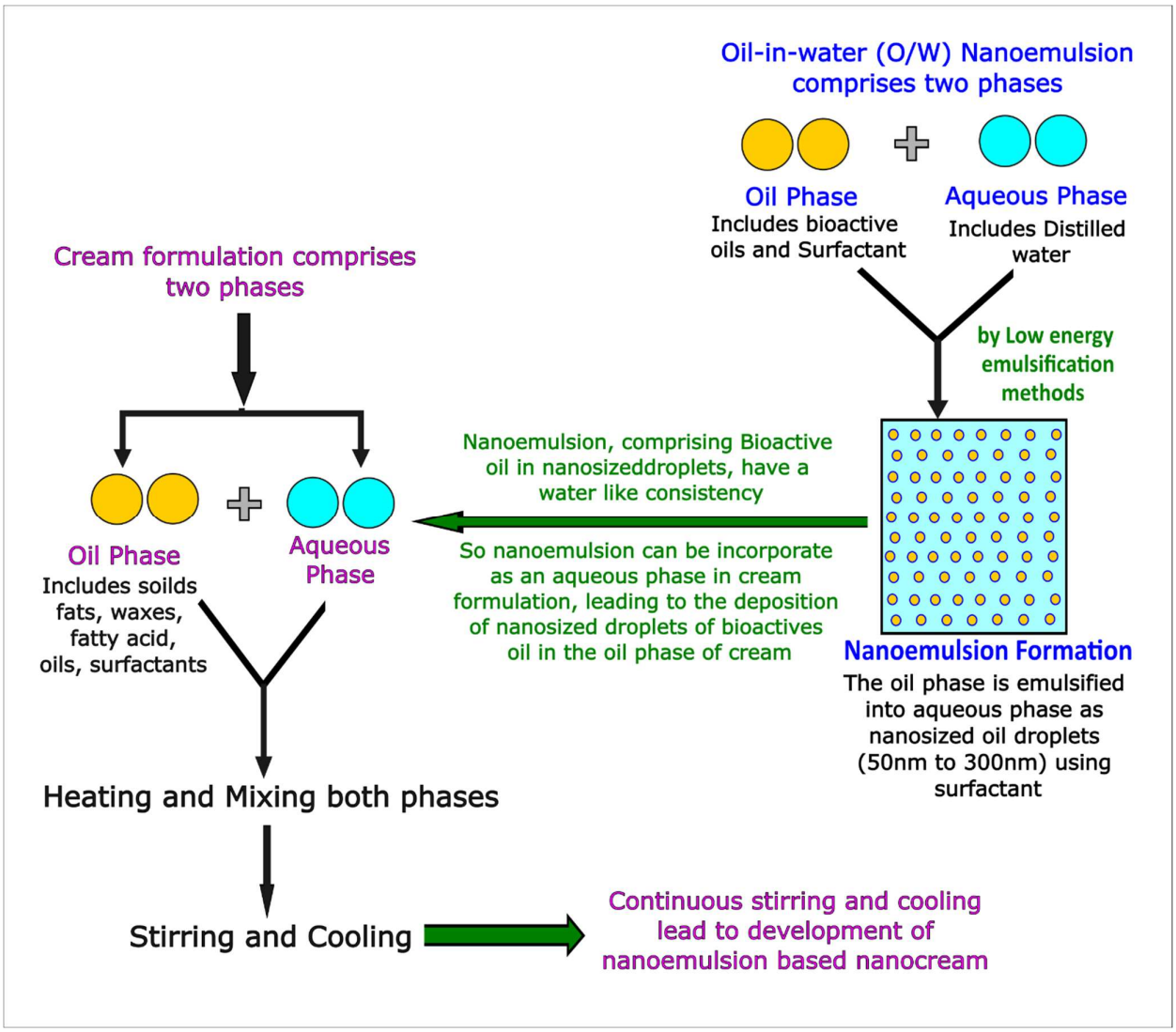

Fig. 1.

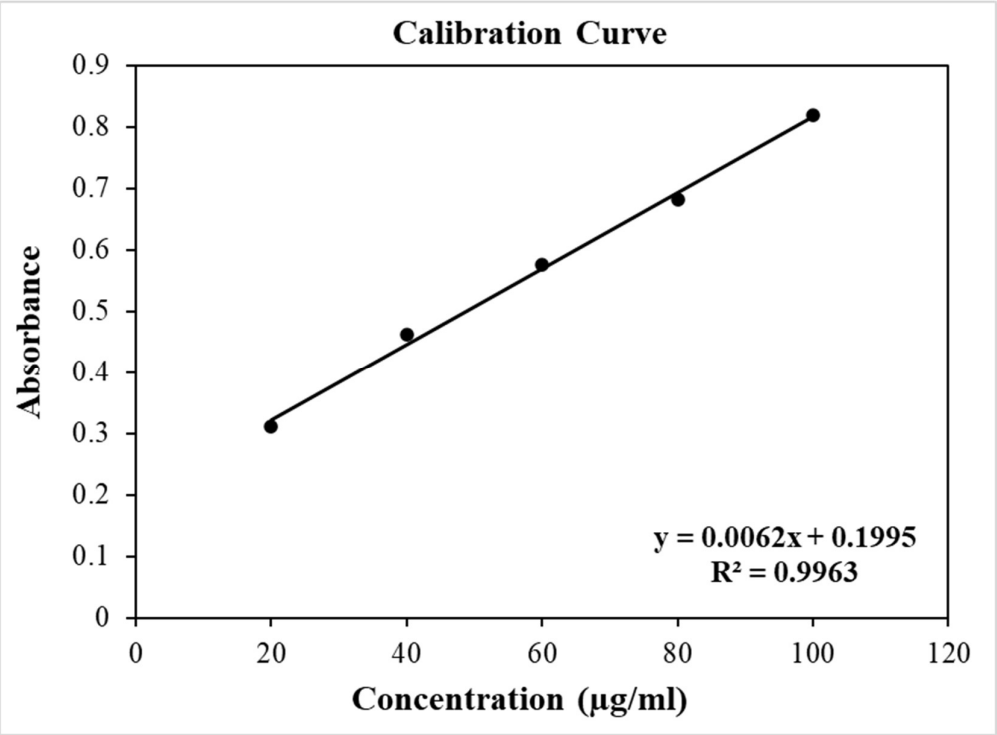

Fig. 2.

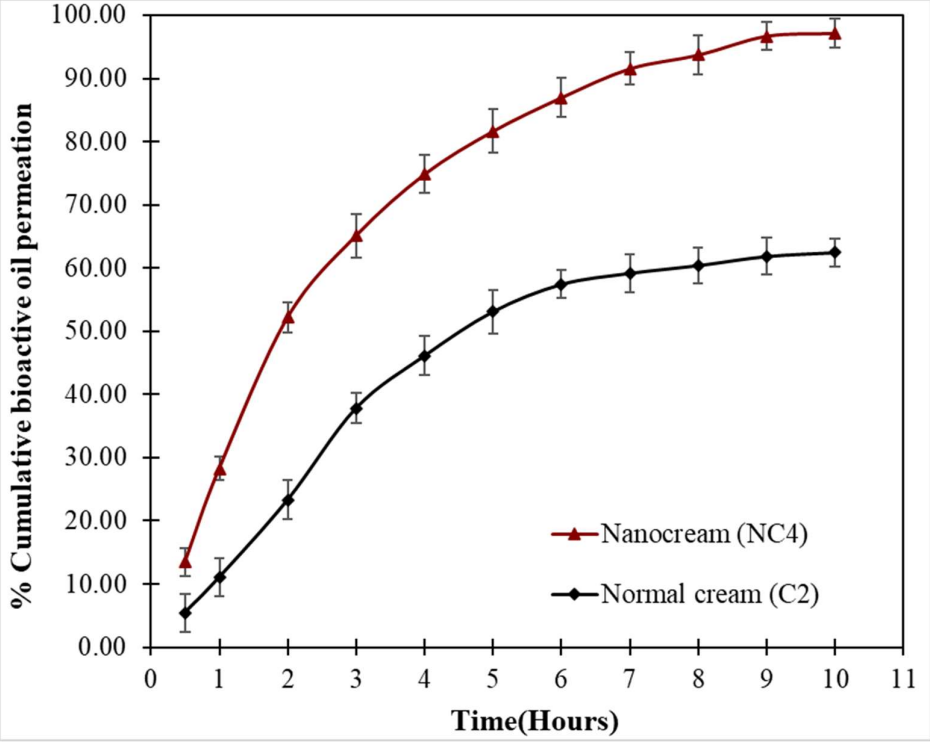

Fig. 3.

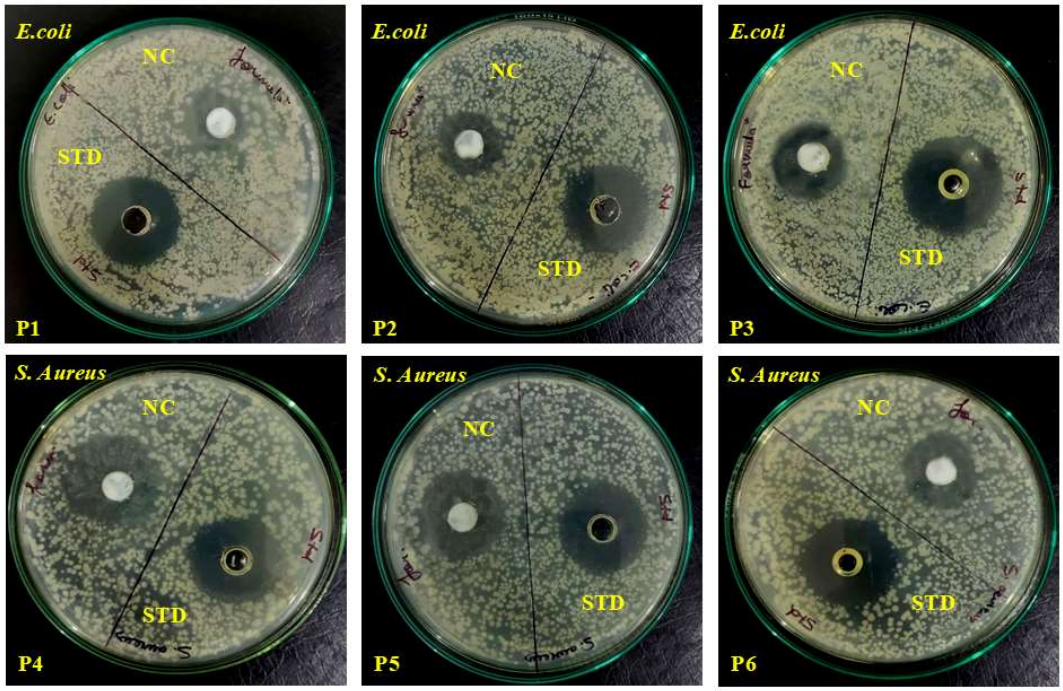

Fig. 4.