

NANOTECHNOLOGY IN COSMETIC FORMULATIONS: RECENT ADVANCES, APPLICATIONS, AND SAFETY PERSPECTIVES

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Abstract

Nanotechnology has proved to be an effective technology in the cosmetic industry, as it has allowed creation of better formulations with enhanced stability, efficacy as well as safety. Nanocosmetics make use of the nanoscale of delivery systems namely liposomes, solid lipid nanoparticles, nanostructured lipid carriers, and polymeric nanoparticles to increase the bioavailability, controlled release and protection of active ingredients. In 2019, it has made great strides in the implementation of nanotechnology in different cosmetic products such as sunscreens, oral care formulations, fragrances, and moisturizers. These nano-enabled systems have been found to be superior in their ultraviolet protection, skin hydration, and longevity of duration of fragrance release, antimicrobial and antioxidant. Besides this, in vitro and in vivo experiments show that designed nanocarriers are well-compatible with skin and have low cytotoxicity. Although these benefits exist, there are long-term safety, environmental, and regulatory standardization issues. This review identifies the current developments in the field of nanocosmetic formulations, attributes the objective advantages and safety issues of such formulations, and the necessity to undertake further research to aid in the responsible and sustainable development of nanotechnology-based cosmetic products.

INTRODUCTION

Nanocosmetics are a category of cosmetic products that use nanotechnology-based materials and the particle size is usually between 1-1000 nanometers, thus improving performance stability, and safety (Nanda et al., 2016). In such formulations, the active ingredients are either engineered on the nanoscale or embedded into nanocarriers of liposomes, solid lipid nanoparticles (SLN), nanostructured lipid

carriers (NLC), polymeric nanoparticles, and nanoemulsions (Viegas et al., 2023). The next introduction of nanostructures, in turn, is a substantial improvement compared to the traditional cosmetic formulations, providing them with a multifunctional processing, without losing aesthetic qualities and consumer comfort (Gupta et al., 2022).

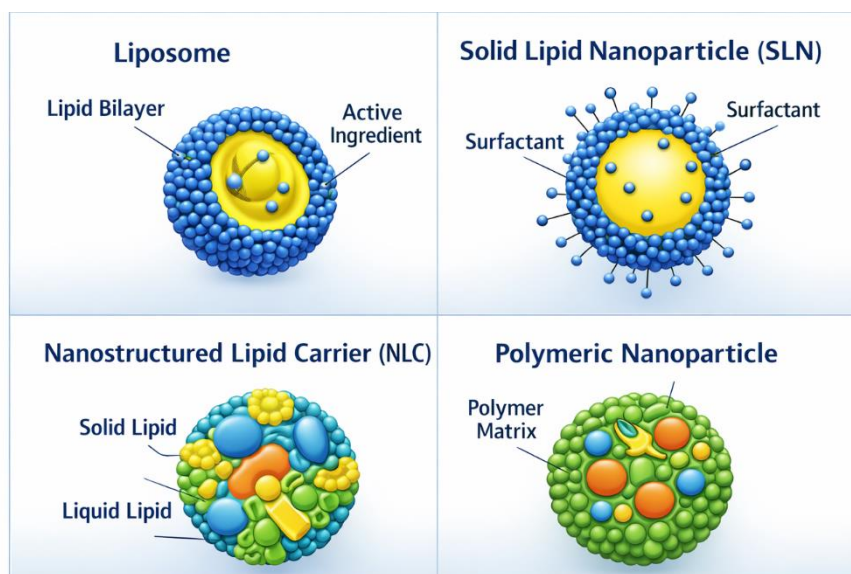


Figure 1. Types of nanocarriers used in nano cosmetics

The nanotechnology is developing in significance within the cosmetic industry due to its ability to overcome many restrictions that are imposed on the conventional cosmetic products (Santos et al., 2019). Traditional recipes often have a low stability, solubility of active ingredients, limited penetrating of the skin, and they degrade easily when coming in direct contact with light, oxygen, or temperature changes (Ioel et al., 2017). These challenges are overcome with nanotechnology-based delivery systems through protecting sensitive ingredients, increasing their physicochemical stability, and providing sustained or targeted release (Mehta et al., 2025). Furthermore, nanocarriers can enhance the level of skin hydration, UV protection, antioxidant action, antimicrobial effects, and the overall performance of the product with a simultaneous decrease in the necessary concentration of active components, subsequently minimizing the possible irritation or toxicity (Vinardell et al., 2015). These strengths highlight nanotechnology as a useful resource in creating safer, more efficient, and innovative cosmetic products (Kaul et al., 2018). The nanocosmetic product research, development, and commercialization have experienced a fast-growing trend in the global cosmetic industry since 2019 (Gupta et al., 2024). The development of nanotechnology in cosmetics has been boosted by the use of nanomaterials, which are green, accessible via

green synthesis, and biocompatible, among other factors, and include the following: sunscreens, moisturizers, oral care, fragrances, and anti-aging formulations (Nhani et al., 2024). This has been further influenced by the growing consumer need to select high-performance and long lasting and environmentally friendly cosmetic products. Also lately, there is a focus on safety consideration, regulation, and social sustainable production behaviors, which is a sign of responsible innovation in nanocosmetics. Altogether, all these achievements demonstrate the fact that nanotechnology is a revolutionary force that will determine the future of the cosmetic business in the post-2019 world (Ferraris et al., 2021).

1.1. Nanotechnology in Cosmetic Formulations

Nanotechnology is central to the current cosmetic formulations due to its ability to formulate sophisticated delivery systems that increase stability, efficacy, and safety (Achagar et al., 2024). The nanoscale materials are characterized by special physicochemical properties, such as high surface area, increased solubility, and better interaction with biological membranes (Zhu et al., 2013). These properties enable cosmetic active ingredients to be effectively transported to the target sites in the skin and prevent degradation of these ingredients by environmental factors such as

light, oxygen and temperature (Casanova et al., 2016). Therefore, cosmetic products that are based on nanotechnology achieve higher performance in comparison to traditional ones.. Liposomes are the type of nanocarriers that have been studied and utilized the most in cosmetics (Ashtiani et al., 2016). The liposomes are spherical vesicles that are made of a single or several phospholipid bi-layers that surround an aqueous center. Their similarity with biological membranes makes them very biocompatible and applicable on the top (Yadav et al., 2017). Both hydrophilic and lipophilic substances can be encapsulated into liposomes, which enhances better penetration into the skin and regulated release of the active components (Dima et al., 2015). Liposomes are extensively used in the cosmetic products in order to provide antioxidants, vitamins, plant extracts and UV filters, increasing skin hydration, protection, and anti-aging properties (Montenegro et al., 2014).

Solid lipid nanoparticles (SLN) represent an important type of nanocarrier that is used in cosmetic effectiveness. These particles consist of lipids, which are kept solid at ambient temperatures and physiological temperatures and stabilized by surfactants (Campos et al., 2020). SLN confer various benefits, among them, increased physical stability, safety of active substances, and profile of release (Kamaly et al., 2016). In the cosmetic industry, SLN are commonly used in sunscreens, moisturizers, and anti-aging products, where they enhance ultraviolet protective factors, enhance cutaneous hydration and give an occlusive effect which helps reduce transepidermal water loss. The solid lipid structure is also an inherent part that adds an extended shelf life and the overall increased stability of the product (Eroglu et al., 2023).

In order to mitigate some of the drawbacks of SLN, especially limiting their drug loading capacity, nanostructured lipid carriers (NLC) were created (Elmowafy et al., 2017). NLC are composite of solid and liquid lipids, which produce a less organized inner scaffolding and can hold a higher number of active ingredients (Salvi et al., 2019). This geometry allows an increase in the efficiency of encapsulation, minimizes the chances of premature release of drugs during storage, and allows more flexible release kinetics. NLC have found a significant focus in cosmetical formulations since they are highly compatible with the skin, possess superior moisturizing properties and are able to deliver both hydrophilic and lipophilic molecules effectively. NLC, in turn, seem especially effective in the long-term skincare use, such as moisturizers and therapeutic cosmetics (Real et al., 2021).

Another popular nanocarrier platform that is extensively utilized in the science of cosmetics is polymeric nanoparticles. This is generally achieved by the creation of such particles using biodegradable and biocompatible polymers, including poly-caprolactone, polylactic acid, and chitosan (Geszke-Moritz et al., 2024). Polymeric nanoparticles allow the active compounds to be controlled and released in a steady way, and they are highly mechanically stable (Elmowafy et al., 2023). They are commonly used to entrap fragrances, antioxidants, antimicrobial agents and plant based bioactives in cosmeceutical preparations. Their ability to enhance the solubility of the hydrophobic compounds as well as their ability to preserve volatile compounds make them particularly useful in perfume and functional cosmetic industries. Moreover, polymeric nanoparticles are involved in the improvement of product serviceability and sensory qualities (Yang et al., 2020).

2. Applications of Nanocosmetics

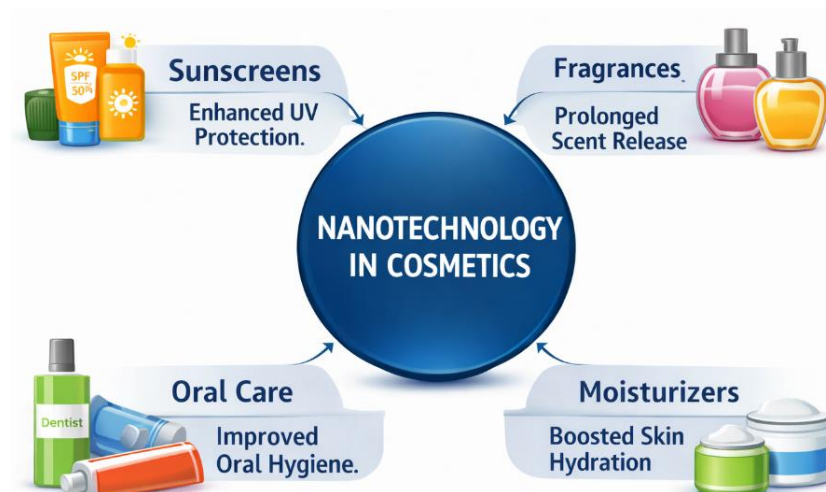


Figure 2: Applications of Nanotechnology in Cosmetics

2.1. Sunscreens

Pavelkova et al. (2020) constructed a new ultraviolet-protective liposomal system that was made of polyhydroxybutyrate (PHB) encapsulating coffee extracts. The coffee extract with a high phenolic content, strong antioxidant capacity and a sun protection factor (SPF) of 40 to 50 was used as a model organic UV filter that had the ability to absorb solar radiation and protect it against transmission to the skin surface. The extract was trapped in liposomal particles which were formulated with lecithin and cholesterol dissolved in deionized water, in the PHB formulation, lecithin was replaced by PHB. All the components were added in the form of chloroform solutions and then sonicated and chloroform was evaporated. In the end, the diameter of the resulting

particles was 140 340 nm as measured using dynamic light scattering and zeta-potential, which indicated the stability through colloidal methods. The cytotoxicity of HaCaT keratinocyte lines was assessed with MTT, lactate dehydrogenase (LDH) assays and all samples showed high encapsulation efficiencies. The 20% PHB inclusion optimized both particle size and stability, increased colloidal stability and also preserved SPF values during prolonged storage. A combination of these PHB-liposome particles up to 12 percent along with coffee extract was considered safe according to LDH testing on HaCaT cells. Since PHB has intrinsic UV-protective capabilities, one can utilize these particles as an effective carrier to organic sunscreen ingredients.

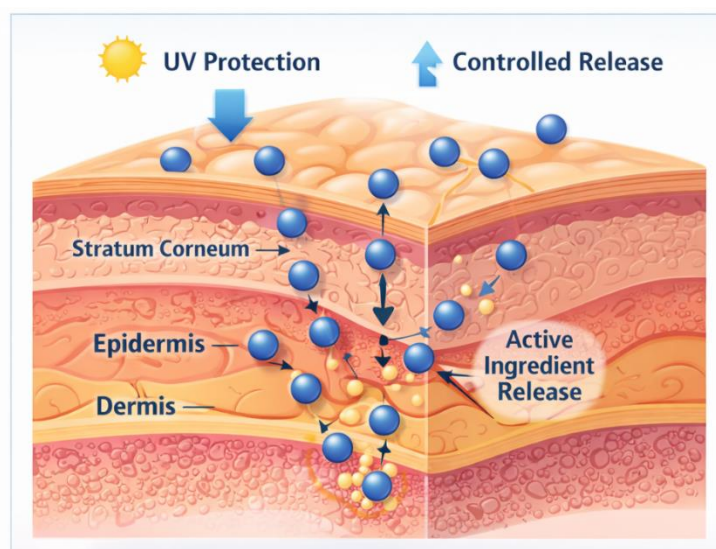


Figure 3: Skin Penetration and Controlled Release of Nano cosmetics

2.1.1. Materials and Methods

Every reagent that was used in this study was of analytical grade. The experimental procedures used deionized and distilled water. *Coffea arabica* beans were extracted using ethanol solution of 20, 40, 60, 80 and 96 percent (American Chemical Society 2016). The quantity of phenolic and flavonoid concentration, antioxidants activity, and SPF were determined using Folin-Ciocalteu reagent, sodium carbonate solution, gallic acid standards, sodium nitrite, aluminium chloride, sodium hydroxide, catechin, ABTS radical, potassium persulfate, and Trolox. Lipid components that were used to prepare liposomes included lecithin and cholesterol. The reagents used in cell culture were Dulbecco Modified Eagle (DMEM) and the following supplements: L-glutamine, fetal bovine serum (FBS), streptomycin, penicillin, phosphate-buffered saline (PBS), sodium dodecyl sulphate (SDS), sodium pyruvate/NADH solution (Asprea et al., 2019). The instruments used included centrifuge, UV-Vis spectrophotometer, probe ultrasonicate, cryogenic transmission electron microscope, zeta-potential analyser. Assessment of cytotoxicity was done using human epidermal keratinocyte (HaCaT) cells lines. The Shapiro Wilk test and one-way ANOVA were used to perform statistical tests.

2.1.2. Extraction and Characterization of Active Compounds from *Coffea arabica*

An extract of active compounds was obtained using aqueous and ethanol solvent system on the coffee beans of the genus *Coffea arabica*. A 1 gram of coffee beans was milled and mixed with 10 mL of an extraction solvent that consisted of deionized water and ethanol in different ratios (20 to 96 per cent). The mixture was then stirred up and down in 24 hours at the ambient temperature to facilitate optimal bioactive constituent extraction success. The obtained extracts were then assigned to physicochemical and biological characterization (Kisoi et al., 2018).

2.1.3. Determination of Total Phenolic Content

FolinCiocalteu colorimetric assay was used to determine the total phenolic content (TPC) of the extracts. The Folin-Ciocalteu reagent was diluted using deionized water and an aliquot was added to distilled water and the extract. After incubating, the saturated solution of sodium carbonate was used to begin the chromogenic development (Lamuela-Raventos et al., 2018). The 750nm absorbance was monitored after 15min by using a UV- Vis spectrophotometer. To construct the calibration curve, gallic acid standards of 0.1 to 0.7mg mL⁻¹ were used. The results are given as mean standard deviation of three independent determinations.

2.1.4. Determination of Total Flavonoid Content

Content of total flavonoid was determined through a modified spectrophotometric method. The extract of a given volume was mixed with sodium nitrite solution and deionized water, and then, aluminum chloride was added to it (Lamuela-Raventos et al., 2024). The reaction was done by the addition of sodium hydroxide and deionized water after incubation. After 10 min absorbance was measured and used as a standard using catechin solutions (0.05-0.37g/ml⁻¹) and calculated the flavonoid concentration by use of the calibration equation (Rashidinejad et al., 2016). All measurements were done in triplicates and in form of mean+SD.

2.1.5. Assessment of Antioxidant Activity

The antioxidant activity was measured based on the ABTS radical-scavenging activity as Trolox equivalents. ABTS radical solution was made by combining ABTS salt and potassium persulfate and letting the solution react in the dark after 12 hours. The solution was diluted with ethanol before it was analyzed and it was made to attain an absorbance of 0.70 at 734 nm. The extract was combined with the ABTS + solution and the reduction of absorbance was noted after 10⁻ minutes (Sun et al., 2018). Quantification was done with a Trolox calibration that has a range of 50–400 ug mL⁻¹. Findings are represented in mean and standard deviation of trio of determinations.

2.1.6. Sun Protection Factor (SPF) Measurement

UV spectrophotometric analysis was done to determine the sun protection factor (SPF) of the extracts. The extracts were diluted with UV Vis ethanol to final concentration of 200 0g mL and absorption spectra were obtained at 290 to 320nm. Ethanol served as a blank. The Mansur equation was used to calculate the SPF values using erythral effect, correlation factor and absorbance with the wavelength. The presentations of the data are in the form of the mean plus standard deviation of three repeat values (Mawaha et al., 2017).

2.1.7. Preparation and Characterization of Liposomal Nanoparticles

Lecithin and cholesterol were used as the lipid constituents to encapsulate coffee extracts into liposomal nanoparticles. The deionized water dispersed lecithin and cholesterol, and chloroform solutions with the extract were added to the mixture. A fraction of lecithin in preparation of PHB based nanoparticles was replaced with polyhydroxybutyrate (PHB). Probe ultrasonication of the mixtures was carried out at a short time, and the solvent was evaporated under regulated heating and magnetic stirring to eliminate chloroform (Wang et al., 2024).

2.1.8. Particle Size, Stability, and Encapsulation Efficiency

A dynamic light scattering method was used to measure particle size and polydispersity index (PDI) as well as colloidal stability with a Malvern Zetasizer Nano ZS. Proper dilution of samples was done before the analysis. Centrifugation of the nanoparticle suspension followed by determination of the total phenolic content in the supernatant was used to determine the encapsulation efficiency. Encapsulation efficiency was also determined as a percentage of the phenolics encapsulated to the total phenolics (Boateng-Marfo et al., 2017).

2.1.9. Imaging of Nanoparticles

The morphology and size of the prepared nanoparticles were examined using cryogenic transmission electron microscopy (cryo-TEM). Surface structure and fiber diameter of PHB nanofibers were further analyzed using scanning electron microscopy (SEM) (Wu et al., 2020).

2.1.10. Cytotoxicity Evaluation

The nanoparticles were tested on human epidermal keratinocyte (HaCaT) cells by the MTT and LDH tests to determine their cytotoxicity. Cells were incubated in the DMEM added with fetal bovine serum, streptomycin, and penicillin under humid and 5% CO₂ at 37 C. Nanoparticle suspensions were filtered, diluted and used on cultured cells. The MTT assay was used to determine metabolic activity, which was calculated as absorbance at 543nm after incubation of MTT and soluble with SDS. The amount of enzyme released into the culture medium was measured using the LDH assay,

which is an indicator of the occurrence of membrane damage, and the absorbance at 540nm was measured (De Almeida et al., 2015).

2.1.11. Statistical Analysis

Each experiment was done in three replicates. The ShapiroWilk test was used to test the data normality, and the significance was tested using the one-way analysis of variance (ANOVA) with a significance level of p less than 0.05. Pearson correlation analysis was done to evaluate relationship among variables and the results were represented as correlation coefficients.

2.2. Toothpaste

Nouri et al. (2022) suggested a nanomaterial green synthesis pathway, which has gained significant scientific interest due to the environmentally-friendly nature and biocompatibility. The use of toothpaste formulations that imply the use of nanoparticles (nanohydroxyapatite) seems to improve the remineralization processes. The nanohydroxyapatite crystals are absorbed into the pores of enamel, serving as the template in the precipitation process, thus, preventing crystal loss and enhancement and refinement of cleaning and whitening properties. Two bacterial species, namely, *Bacillus subtilis*, and *Bacillus coagulans*, were grown in a medium with insoluble calcium phosphate; the organisms synthesised nanohydroxyapatite and nanocalcite. They purified the nanoparticles using the filtration process with the use of a 200nm membrane and heat treatment. Fourier transform infrared spectroscopy, X-ray diffraction, scanning electron microscopy, ultraviolet-visible spectroscopy, energy-dispersion X-ray analysis, and X-ray fluorescence were used to perform structural and compositional characterisation. Sublimation of insoluble calcium phosphate in the presence of urea has been proven to be another step requiring to be followed in the synthesis of nanohydroxyapatite because in this process phosphatase and urease activity are induced. Nanohydroxyapatite particles obtained are less than 100nm in size, are bio-produced, and therefore non-toxic and can be used in food, toothpaste, and washing products. The reason is that braid hydroxyapatite can substitute the

needle-like food additives in infants and the elderly because it has a good safety profile.

2.2.1. Materials and Methods

All the reagents and materials that were used in this experiment were of an analytical quality. Nanoparticle biosynthesis and bacterial growth were done using nutrient agar, dextrose, tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$), sodium chloride, potassium chloride, magnesium sulfate heptahydrate, yeast extract, ferrous sulfate heptahydrate, and manganese sulfate monohydrate. The culture media was solidified using agar, and p-Nitrophenyl phosphate was used as the enzyme assay substrate (Meghana et al., 2020). The synthesized nanoparticles were characterised using a combination of analytical tools, such as, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), ultraviolet visible near infrared spectrophotometry (UV Vis NIR), energy dispersive x-ray spectroscopy (EDX), and X-ray fluorescence (XRF) spectroscopy (Mohan et al., 2016). Other required equipment included a furnace with sintering, microcentrifuge, spectrophotometer to make quantitative analysis.

2.2.2. Cultivation of Bacteria and Biosynthesis of Nano-Inorganic Materials

The strains of bacterial probiotics that were utilized in the production of nano-inorganic materials were standard strains of *Bacillus coagulans* ATCC 7050 and *Bacillus subtilis* PTCC 1204. First, the two strains were separately cultured on nutrient agar plate after a heat-shock treatment and incubated at 37 °C in 24 h. Afterwards, all of the strains were inoculated on Pikovskaya (PVK) agar media in the presence of 0.1% urea supplementation (Nouri et al., 2022). The PVK medium was composed of dextrose (10 g L⁻¹), tricalcium phosphate (5g L⁻¹), sodium chloride (0.2g L⁻¹), potassium chloride (0.2g L⁻¹), magnesium sulfate heptahydrate (0.1g L⁻¹), yeast extract (0.5g L⁻¹), ferrous sulfate heptahydrate (0.002g L⁻¹), manganese sulfate monohydrate (0.002g L⁻¹). The capacity of the bacterial strains to form calcite and hydroxyapatite was measured by seeing transparent colonies on the PVK agar plates which indicate solubilisation of insoluble tricalcium phosphate through phosphatase

activity. The incubation duration was five days, and bacterial biomass was collected on the agar plate and dried under the room temperature (25 \pm 3 \circ C) on the sterile plates in an hour. The synthesis of nanoparticles was carried out in three steps, namely the formation of particles in the dry bacterial surface, sintering of the dried biomass in the presence of 600 \circ C at 2 hours, and lastly purification of hydroxyapatite nanoparticles by means of nanofilters. The resulting dried and sintered powders were further characterised (Monroy Miguel et al., 2020).

2.2.3. Identification and Characterization of Nano-Inorganic Particles

It was determined that the crystalline structure of the synthesized nanoparticles could be studied with the help of the X-ray diffraction at 35 kV and 30 mA in the reflection mode applying the CuK alpha radiation (length of the wavelength 1.540598 Å). The patterns of XRD were observed at 2 θ of 10-80 \circ with a scanning speed of 0.04 \circ /s and before and after purification. Fourier transform infrared spectroscopy was used to analyse the nanoparticles in terms of functional-group analysis in the 350-4000 cm^{-1} . The morphologies of the surface and structure of particles were observed under scanning electron microscopy at an accelerating voltage of 20 kV after gold sputtering with a 15 nm coating. Optical properties were assessed by means of diffuse reflectance UV-Vis spectroscopy under 220-2200 nm (Nouri et al., 2022).

2.2.4. Assessment of Calcium-to-Phosphorus Ratio

The energy-dispersive X-ray spectroscopy and X-ray fluorescence spectroscopy were used to find out the elemental composition and calcium to phosphorus (Ca:P) ratios of the synthesized nanoparticles. All the samples were gold-coated before analysis. To verify the chemical structure and purity of the produced nano-inorganic materials, XRF measurements were conducted to elemental profile all the way down to uranium (Alaneme et al., 2025).

2.2.5. Enzyme Activity Assays

Enzyme activity alkaline phosphatase (ALP) was used to determine the contribution of bacterial

metabolism in the formation of nanoparticles. Five days of cultures of bacterial strains in PVK broth and PVK broth enriched with urea and nutrient broth were carried out. Centrifugation of cultures was done at 4000 g for 20 minutes after which cell-free supernatants were removed to further analysis of the enzyme. The activity of ALP was identified by incubating the supernatant with a phosphatase substrate solution of 100mM Tris buffer (pH 10) and 7.6mM p-nitrophenyl phosphate at 37 \circ C during one hour. A spectrophotometer was used to measure absorbance at 405nm at the end of the reaction by the addition of 0.5M sodium hydroxide. The defined unit of enzyme activity was the quantity of enzyme that liberates 1.0 mmol of p-nitrophenol every minute under conditions of assay (Chen et al., 2022).

The urease activity was assessed by the use of urea agar media. The bacteria that degraded urea generated ammonia as it grew and this led to the alkalisation of the medium and the colour of the phenol red indicator changed to pink. To make experiments reproducible, all of them were done in triplicates (Ojha et al., 2025).

2.3. Perfumes

The authors have overcome this shortcoming by synthesising the polycaprolactone nanoparticles that encapsulate geraniol and Palmolive essential oil. Encapsulation makes the hydrophobic constituents release under controlled release and improve solubilisation in hydrophilic media. The constitutive oil was obtained through steam stripping through a Clevenger extractor. Analysis by GC-MS showed that geraniol was 70.27% of the oil. Follow-up analysis by HPLC determined that 6.80mg geraniol was present in 100mg essential oil and this demonstrated that liposomal encapsulation enhanced the stability and solubility of geraniol. Liposome integration was especially efficient with components that were insoluble in aqueous. In the case of geraniol and Palmarosa oil, the average diameter of loaded nanoparticles was 282.1 nm in comparison with 289.3 nm in the case of the free-loaded nanoparticles. Changes in the type of polymer used, concentration in the organic phase, solvent polarity, ratio of internal to external phase, surfactant concentration, and essential oil loading had an impact on particle size. The

polydispersity index also proved the homogeneity of the size distribution (Lammari et al., 2020). The stability of nanoparticles in storage and unimodal dispersion were exhibited by values of PDI less than 0.140. A Zeta potential value of less than 30 mV was a sign of good colloidal stability (Masarudin et al., 2025). It was found that it had high encapsulation efficiency, which is due to the hydrophobic characteristics of geraniol and the essential oil. Onset and endpoint temperatures were validated by the shift in temperature changes using the differential scanning calorimetry as a result of encapsulation. The nanoparticles were antioxidant and were active against DPPH radical and inhibited *Escherichia coli* and *Staphylococcus aureus*. These results indicate that the polycaprolactone nanocapsule has favourable physicochemical properties and it could be used in the fragrance, cosmetic, and pharmaceutical industries (Chiriac et al., 2021).

2.3.1. Materials and Methods

All reagents and chemicals used were of analytical quality. The polymeric nanoparticles were prepared using Poly ϵ caprolactone (PCL), Pluronic F68, geraniol, and lecithin (S 75 3) (Patel et al., 2022). The organic solvents used in nanodispersal were acetone and ethanol. Through a Clevenger apparatus, palmarosa essential oil was obtained through steam distillation. The analytical and characterisation instrumentation included gas chromatography (GC)-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), dynamic light scattering (DLS), differential scanning calorimetry (DSC), and transmission electron microscopy (TEM). Antioxidant activity was measured on ethanol and DPPH radical solutions, whereas the antimicrobial activity was measured against *Staphylococcus aureus* (ATCC 2593) and *Escherichia coli* (ATCC 25922) growing in brain-heart infusion (BHI) broth. The procedures were followed by protocols of standard laboratory centrifugations and spectrophotometrics (Antunes Filho et al., 2023).

2.3.2. Extraction and Quantification of Palmarosa Essential Oil

Leaves of palmarosa were dried at 45 °C, before taking out essential-oil. The oil was made by steam distillation, which was performed using a Clevenger apparatus. The GC-MS with ZB-5MS capillary column (30 m x 0.25 mm x 0.25 mm) was used to characterize the chemical composition. The injector temperature was kept at 250 °C with helium being the carrying gas at 1.0 mL/min. Oven programme was started at 60 °C and then 210 °C and then maintained to run a 35 minutes run (Kakaraparthi et al., 2015). Oil samples were injected into the column after being diluted 200-fold by hexane of chromatographic grade. The identification of the components was performed using mass-spectral libraries (NIST-05) and reported retention indices; quantification was performed based on the normalised peak areas in the total ion chromatogram (Smitha et al., 2015).

According to the identified leading GC-MS constituent, the geraniol concentration of Palmarosa essential oil was determined through HPLC. The photodiode array detector and BDS-Hypersil C18 analytical column were used in the analysis under a guard column protection (Roy et al., 2022). The mobile phase was a mixture of methanol and water (75:25 v/v) at 1.0 mL/min. The column was run at 50 °C and the injection volume was 20 μ L at 275nm. The standard solutions ranging between 0.01 and 0.08 mg mL⁻¹ were used as the calibration curves, and the information was analyzed with the help of chromatographic work-station software (Luo et al., 2020).

2.3.3. Formulation of Polymeric Nanoparticles

The nanoprecipitation technique was used to produce polymeric nanoparticles that contain geraniol or Palmarosa essential oil. An acetone solution containing 35 Mg mL of PCL was mixed with a 60:40 v/v solution of acetone and ethanol containing lecithin. The PCL solution was pre-precipitated with geraniol or Palmarosa oil. The organic phase was then resuspended in an aqueous solution of Pluronic F68 (1 per cent m/v) by magnetic stirring at 25 °C (Adeyemi et al., 2023). The presence of immediate milky suspension meant that nanoparticles were generated. The suspension was then filtered on an 80 nm porosity filter and stirred at ambient



temperature to eliminate any remnants of organic solvents. Recipes were made NPT-free (no encapsulated agents), NPT-G (geraniol-loaded nanoparticles), and NPT-EO (Palmarosa essential-oil-loaded nanoparticles) (Jummes et al., 2020).

2.3.4. Nanoparticle Characterization

The efficiency of the encapsulation was ascertained by centrifugation at 6000 rpm in 30 minutes and the usage of ultrafiltration membranes with a cut-off of 30Kaa. Encapsulated compounds were retained on the membrane and the unencapsulated ones were measured by HPLC under the aforementioned chromatographic conditions. The efficiency of the encapsulation was determined as a percentage of the encapsulated material to the original load (Marques et al., 2020).

Dynamic light scattering was used to measure the average particle size (Z -average), polydispersity index (PDI), and the zeta potential (ζ). A Zetasizer Nano Series instrument was used to measure these. Before measurement, samples were diluted with an equal amount of distilled water (2:1 (v:v)). The reaction studies were performed at 25 °C under the scattering angle of 173 and the data analyzed with Zetasizer software (Yeap et al., 2018).

Differential scanning calorimetry (DSC) was used to determine the thermal properties of the nanoparticles. A mini spray dryer dried samples of nanoparticles at 130 °C, pump setting controls, and aspiration setting controls. About 10 mg of the dried samples was put in aluminum pans and heated between 25-120 °C at 10 °C/min in the presence of nitrogen (de Oliveira et al., 2013).

The morphological characterization was done using transmission electron microscopy (TEM). Nanoparticles suspensions were diluted using ultrapure water, and the aliquots were deposited on carbon-coated copper-grids. The grids were dried at room temperature and observed under a JEOL TEM at a 70 kV (Minocha et al., 2012).

2.3.5. Antioxidant Activity

The DPPH free radical scavenging assay was used to determine the antioxidant activity of the nanoparticle formulations. A suspension of nanoparticles was prepared with ethanol (85% v:v), sonicated over 30 minutes and left at rest over 15 hours. The nanoparticle extract reacted

with DPPH solution (0.1mmol/L $^{-1}$), and both were left in the dark and incubated at 24h. The level of absorbance at 515 nm with a UV-Vis spectrophotometer was used to measure the level of antioxidant activity, which was in the form of the percentage of inhibition of the DPPH radical (Aldayel et al., 2023).

2.3.6. Antimicrobial Activity

Free and encapsulated formulations were tested against the antimicrobial activity of the bacteria: *Staphylococcus aureus* (ATCC 2593) and the bacterium *Escherichia coli* (ATCC 25922). The growth of bacterial cultures was in braininfusion-heart broth and adjusted to about 10^3 and 10^6 CFU mL $^{-1}$, respectively. BHI agar plates and 6mm diameter wells were aseptically prepared with the inoculated cultures being spread into them. Nanoparticles suspensions were introduced into the wells and plate was pre incubated at 4 °C of wells with 2 h of diffusion and incubated at 37 °C of wells with 24 h incubation. The levels of antibacterial activity were determined by the measurement of the diameter of the inhibition zones around every well (Jayathilaka et al., 2022).

2.3.7. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean \pm standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with significance considered at $p < 0.05$.

2.4. Moisturizers

As Pereira-Leite et al. (2023) state, the outer layer of the skin in this case, stratum corneum, is considered a defence against xenobiotics, and any changes in the lipid matrix also relate to cutaneous dysfunctions. The contribution of ceramides to the skin barrier is important but free fatty acids like stearic acid are also significant. SLN and NLC based on stearic acid and glycerol fatty acid were prepared to investigate both of them as primary lipid carriers. A number of stearic-acid blends, Precirol-ATO-5, Capryol-90, and Tween-80 were tested in this regard. The process of obtaining SLN was performed by mixing in a water bath (TERMOFIN, JP Selecta, Spain) solid lipids (Precirol 5 ATO and/or stearic acid) and a

surfactant (Tween 80). The aqueous phase was then stirred with 10^{-1} in an equal volume of the lipid phase to form the lipid phase. The sonication was also done in 5 min at 70 percent amplitude with an ultrasonic sonicator (Q125 ultrasonic sonicator, Qsonica Sonicators, Newtown, CT, USA). The nanoparticles would be solidified by afterward cooling at room temperature.

The NLC preparation process was similar to that of SLN, only that the liquid lipid (Capryol 90) was added to the lipid phase before heating at 75 °C. Dynamic light scattering was used to analyze the particle size and PDI, and pH was sampled through a pH meter, and viscosity was sampled through a rotational viscometer (H Muller et al., 2011). These parameters evaluated stability of the nano-system, biological performance and release properties of the nano-system. The pH of the nanoparticles was suitable to be used on the surface. It is important to note that the NLC3 formulation had a low pH than the SLN3. Lipid nanoparticles were typically of low viscosity with exception of NLC-3. In general, the results showed that the majority of formulations were not degraded by mechanical and thermal forces (Asad et al., 2021).

To determine safety and efficacy in 90 days, as well as accelerated and long-term stability, in vivo studies were carried out. Topical administration with nanoparticles of size less than 300nm, PDI less than 0.3, skin-compatible pH, and viscosity less than 5mPas showed promising properties of topical administration (Qaiser et al., 2024). SLN2 and NLC2 concentrations showed very high stability in the long run. The application in an open or occluded condition did not show any adverse effects when tested in human voluntary biocompatibility (Makoni et al., 2019). It was found that the most stable Precirol AT O 5 nanoparticles that have been used in efficacy studies have enhanced skin hydration. The lipid assemblies used in this study exhibited high biocompatibility and moisturising properties, and obtained nanoparticles can be used in cosmetic applications (Pereira-Leite et al., 2023).

2.4.1. Materials and Methods

All the materials used in this study were of an analytical grade. The lipid constituents were stearic acid, Tween 80, Capryol 90, and Precirol

Ato 5 that were used to prepare solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) (Jafar et al., 2021). A water bath was used to control the heating used in the preparation of nanoparticles. The size of the particles and index of polydispersity were measured using a Delsa Nano C Particle Analyzer that has a dual 30 mW laser. Physicochemical properties of the resulting formulations were also studied using a calibrated pH meter and rotational viscometer. The Corneometer CM825 was used to measure the skin hydration and the patch tests were used to conduct in vivo measurements using the occlusive patches. GraphPad Prism 8.0.2 was used to carry out statistical tests employing the one-way and two-way analysis of variance (ANOVA) (Danaei et al., 2018).

2.4.2. Preparation of Lipid Nanoparticles

The solid lipid nanoparticles were prepared by melting a blend of solid lipids, in this case, Precirol in a water bath at 75 °C and/or stearic acid in addition to the Tween in 80. At the same time, the lipid phase was heated in a double-distilled water and then it was mixed into a single mixture. The emulsion that developed was ultrasonicated at 70 per cent amplitude over 5 minutes to decrease the particle size and create a nanoemulsion (Flemban et al., 2022). This nanoemulsion was then left to cool to the room temperature, and the nanoparticles solidified. The preparation of nanostructured lipid carriers was done following the same procedure with the difference that the liquid lipid Capryol 90 was added to the lipid phase before heating was done at 75 °C. Six unique formulations were made in triplicates to make the formulations reproducible (Viegas et al., 2023).

2.4.3. Physicochemical Characterization of Nanoparticles

Dynamic light scattering was used to determine the size of the particle and the polydispersity index of the prepared formulations on the Delsa Nano C Particle Analyzer at 165 °C scattering angle. Before being measured, nanoparticle suspensions were diluted 50-fold using distilled water to reduce interference of viscosity and multiple scattering. The formulations were tested at 70 measurement cycles and triplicated (Razafindralambo et al., 2019). A calibrated pH

meter was used to measure the PH of the formulations at room temperature. The viscosity was measured using a rotational viscometer that had concentric cylinders. They were analyzed under the controlled temperature conditions (25 °C) and with the help of suitable spindles and rotation speed parameters based on the type of formulation and all the results were performed in triplicates (Shukla et al., 2018).

2.4.4. Stability Studies

The accelerated stability tests were done to replicate the stress conditions and determine physicochemical stability of the formulations. Two supplementary strategies were used. The former entailed centrifugation of formulations at 3000 rpm /30 min to determine possible phase separation and physical instability. The second method was a gradual temperature rise experiment, whereby the formulations were heated in a water bath at 40 °C and heated up at 10 °C per 30 minutes at a time up to 80 °C. The formulations were subsequently assessed in terms of organoleptic, pH, and viscosity, as well as thermal instability visible indicators after being cooled to room temperature (Kashinath et al., 2024). The test of long-term stability was done by keeping the formulations in amber glass vials at room temperature (25 °C) at 90 days. Physicochemical characterization such as particle size, polydispersity index, PH and viscosity were carried out at fixed time intervals of day 7, 15, 30, 60 and 90. The visual inspection was also conducted to identify precipitation, phase separation, or the presence of microbial contamination (Crommelin et al., 2018).

2.4.5. In Vivo Skin Compatibility Assessment

The female volunteers in the age group of 21 to 55 years were sampled in skin compatibility studies and the relevant institutional ethics committee and all the participants provided written consent to participate in the studies. Each volunteer received seven test sites which were identified within the volar forearms to denote three SLN formulations, three NLC formulations and an untreated area. The preparations were used in two days (one time per day) and the dosage was 2 mg/cm² utilizing a randomized application plan. Data was collected using biomaterials that were relevant to the

determination of skin color, Trans epidermal water loss, and stratum corneum hydration, which included a chromameter, a Tewameter TM300, and a Corneometer CM825. Measures were taken during the baseline before the application, and subsequent inspection was performed after 24 and 48 h of treatment (Hertz-Kleptow et al., 2023).

Single-application patch test of the occlusive with a single application was also conducted to determine the possible skin irritation. The formulations were administered under occlusion after 24 hrs at the lower back of the volunteers using Finn Chambers 24 hrs on the lower back of the volunteers and distilled water was used as the control. Visual scoring on the skin reactions was also performed by the same researcher, after patch removal, to reduce observer bias (Wattanakrai et al., 2007).

2.4.6. Efficacy Assessment

The effect of formulations in moisturizing was assessed in another study in healthy male and female volunteers between 9 and 48 years old. The volar forearm of each volunteer was marked with 3 test sites and one untreated control site; one test site was applied to with the SLN2 and another test site applied to NLC2 formulations. At a volume of 200 µl, using an occlusive patch, the formulations were applied on 4 h since they had low viscosity. The moisturizing performance of the formulations was assessed by measuring the stratum corneum hydration in the presence of the formulation using the Corneometer CM825 before and after application (Brooks et al., 2016).

2.4.7. Statistical Analysis

All the experimental results were expressed in mean standard deviation. GraphPad Prism 8.0.2 software was used to make statistical analyses. Accelerated stability information involved one-way ANOVA with subsequent multiple comparison test by Tukey, whereas the charismatic and efficacy studies of nanoparticles involved application of Sidak multiple comparison test. Two-way ANOVA with Dunnett as a post-hoc test were used in the analysis of data acquired in the skin compatibility and long-term stability research. The statistical significance of differences was assumed at p less than 0.05 (Eiras et al., 2017).

Conclusion

Nanotechnology has contributed a great deal to the development of cosmetic formulation in terms of enhancing the stability, delivery and efficacy of the active ingredients. The application of nanocarriers i.e. liposomes, solid lipid nanoparticles, nanostructured lipid carriers, and polymeric nanoparticles has facilitated better skin penetration, controlled release and better performance of the products. The performance and versatility of nanocosmetic systems is shown in recent uses of these systems in sunscreens, oral care, fragrances and moisturizers. Even though it is found that the studies are safe and biocompatible, research and stepwise evaluation is needed to guarantee long-term safety and sustainable development. Altogether, nanotechnology has high potential that can define the future of innovative and high-performance cosmetic products.

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