RESEARCH PAPER

Development and characterization of caffeine-loaded nanostructured lipid carriers incorporated into a hyaluronic acid-based gel for enhanced topical skin delivery

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ABSTRACT

Objective(s): The current study focused on the formulation and physicochemical characterization of nanostructured lipid carriers (NLCs) containing caffeine incorporated into a hyaluronic acid (HA) gel for enhanced topical skin delivery. The approach focused on improving caffeine encapsulation, stability, and localized skin accumulation.

Materials and Methods: The NLC formulations were prepared using high-shear homogenization and probe sonication methods. Their hydrodynamic diameter, polydispersity index (PDI), and surface charge were evaluated by dynamic light scattering, while their morphological features were observed using field-emission scanning electron microscopy (FE-SEM). Drug–excipient compatibility and caffeine's physical state in the lipid matrix were assessed via FT-IR and DSC. Encapsulation efficiency (EE %) was determined spectrophotometrically. Ex vivo skin permeation and deposition were studied on excised mouse skin using Franz diffusion cells, compared with a conventional formulation.

Results: After optimization, the NLC formulation had a particle size of 240.5 ± 4.7 nm, a PDI of 0.177 ± 0.020, and a zeta potential of −8.09 ± 1.3 mV, indicating a homogeneous, stable colloidal system. EE% was 77.1 ± 0.6%. FE-SEM showed spherical, smooth particles. FT-IR and DSC showed no chemical interactions and suggested that caffeine was molecularly dispersed within the lipid core. Ex vivo studies demonstrated increased skin retention and reduced transdermal permeation compared to conventional formulations. Incorporating NLCs into the HA gel further enhanced dermal localization, highlighting a synergistic effect between the lipid carrier and hydrophilic polymer.

Conclusion: The caffeine-loaded NLC-HA gel is a stable and efficient topical delivery system. It offers high encapsulation efficiency, sustained release, and superior dermal retention of caffeine with minimal systemic absorption, providing a promising platform for enhanced topical caffeine delivery in skin applications.

Keywords: *Caffeine; Nanostructured lipid carriers; Topical administration; Skin absorption; Hyaluronic acid*

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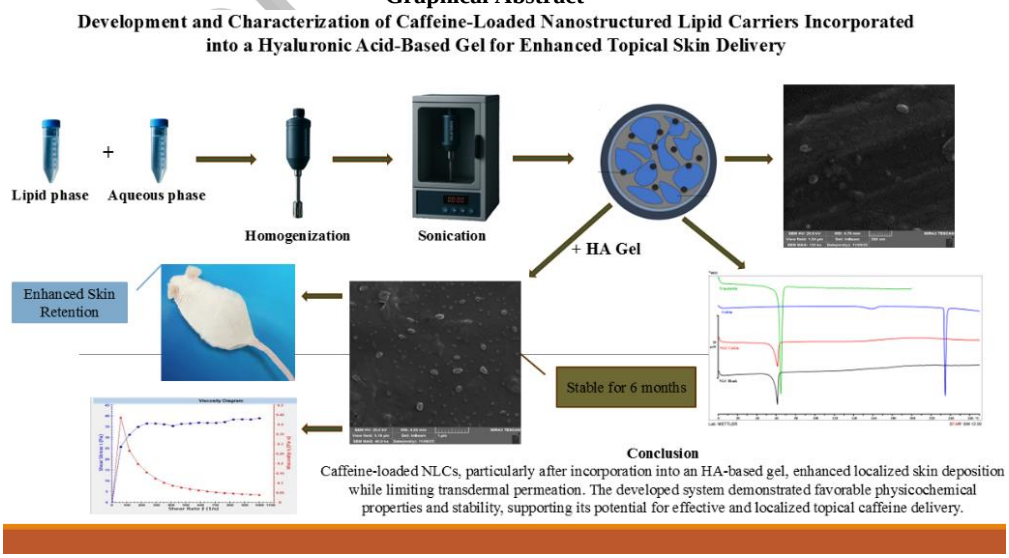
Graphical Abstract



Caffeine-loaded NLCs incorporated into a HA-based gel for enhanced localized skin delivery

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INTRODUCTION

As the body's largest organ, the skin provides an essential protective interface with the external environment and plays a central role in maintaining physiological homeostasis. Beyond its barrier properties, adequate skin hydration and microcirculation are essential for maintaining skin integrity, appearance, and function. Disruption of these processes can lead to common clinical manifestations such as dryness, reduced elasticity, uneven skin tone, and premature aging. These changes are often related to reduced barrier function and increased transepidermal water loss, highlighting the need for effective topical delivery strategies to improve skin condition and performance [1, 2].

Caffeine is a naturally derived methylxanthine alkaloid that has attracted considerable interest for topical use because of its range of biological effects. Among these, its vasomodulatory activity may contribute to improved local microcirculatory function [3, 4] as well as lipolytic activity through phosphodiesterase inhibition and cAMP elevation, contributing to localized fluid reduction [5]. Caffeine also exhibits antioxidant activity, partly through free-radical scavenging and regulation of oxidative stress-related pathways, which further supports its incorporation into cosmetic and dermatological formulations [6-8]. However, its hydrophilic nature and limited affinity for the lipid-rich stratum corneum restrict its skin penetration and reduce its efficacy in conventional formulations [9, 10].

Nanostructured lipid carriers (NLCs) have consequently gained attention as alternative lipid-based platforms for overcoming some of the limitations related to conventional delivery systems. Their formulation characteristics can provide greater drug-loading capacity, modulate drug release, and improve physical stability compared with traditional lipid nanoparticle systems. Their mixed solid–liquid lipid matrix creates structural imperfections that enhance drug incorporation and reduce drug expulsion during storage [11]. For caffeine, NLCs may improve skin retention and localized delivery by modulating release kinetics and enhancing deposition within superficial skin layers.

Hyaluronic acid (HA) is a naturally occurring biopolymer widely used in topical formulations due to its strong water-binding capacity and moisturizing properties. When formulated as a topical gel, HA can improve skin hydration and serve as a suitable vehicle for topical delivery systems. Moreover, HA gels provide favorable rheological properties that may enhance formulation stability and user acceptability. However, its combination with lipid-based nanocarriers remains relatively underexplored [12-14].

Although caffeine-loaded NLCs have been previously investigated, their incorporation into HA-based gel systems has received limited attention. Furthermore, systematic optimization of such combined systems with emphasis on improving skin deposition and overcoming the inherent limitations of caffeine delivery remains insufficiently explored. In this context, integrating NLCs into an HA gel matrix may provide synergistic benefits by combining enhanced encapsulation and controlled release with improved hydration and formulation stability [15, 16].

Therefore, the novelty of this work lies in the development and optimization of a caffeine-loaded NLC incorporated into a HA-based gel as a topical delivery system, focusing on improving encapsulation efficiency, stability, and skin deposition to overcome the inherent limitations of caffeine delivery.

The investigation was conducted in sequential stages, beginning with the preparation of caffeine-loaded NLCs followed by their characterization with respect to particle size, polydispersity, morphology, encapsulation efficiency, and stability. The optimized NLC formulation was subsequently incorporated into an HA-based gel to obtain the final topical system. The formulation was then evaluated for in vitro drug release, and its skin delivery potential was further assessed using an ex vivo rat skin permeation model in comparison with conventional formulations.

MATERIALS AND METHODS

Materials

Caffeine and medium-chain triglyceride (MCT) oil were obtained from Sigma-Aldrich (Germany). Compritol® 888 ATO and Precirol® ATO 5 were kindly provided by Gattefosse (France), while Tween® 80 was purchased from Uniqema (Belgium). Cetyl palmitate, tripalmitin, and vitamin E (α-tocopherol) were obtained from Merck (Germany).

Optimization and preparation of caffeine-loaded NLCs

A preliminary screening was first performed to qualitatively assess the solubility of caffeine in the selected lipid and surfactant components. The solid lipid candidates included Precirol® ATO 5, Compritol® 888 ATO, cetyl palmitate, and tripalmitin, while MCT oil was evaluated as the liquid lipid and Tween® 80 as the surfactant.

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Caffeine-containing NLC dispersions were produced by combining high-shear homogenization and probe sonication. The lipid phase, including the selected solid lipid, MCT oil, caffeine, Tween® 80, and vitamin E, was heated separately from the aqueous phase (deionized water) to 80°C. The preheated aqueous phase was subsequently added to the lipid phase while homogenizing at 12,000 rpm for 5 min using an Ultra-Turrax T25 homogenizer (IKA, Germany). The resulting dispersion was further processed by probe sonication for seven cycles (60 s ON/15 s OFF). The preparation was then permitted to cool to room temperature, resulting in the formation of the NLC dispersion. Caffeine concentrations between 0.1% and 3% (w/v) were examined during the initial formulation screening [17].

Particle size, PDI, and zeta potential

The hydrodynamic particle diameter, PDI, and zeta potential of the NLC dispersions were evaluated by dynamic light scattering using a Zetasizer Nano-ZS (Malvern Instruments, UK). Before determination, the samples were diluted 100-fold (1:100, v/v). Measurements were conducted at 25°C, with three independent measurements performed for each sample.

Encapsulation efficiency (EE %) measurement

The amount of non-encapsulated caffeine was used to indirectly determine the encapsulation efficiency of the NLC formulation. A 500 µL aliquot of the nanoparticle dispersion was placed in an Amicon® Ultra centrifugal filtration device fitted with a 100 kDa MWCO membrane. Centrifugation was performed at 14,000 rpm for 10 min at 4°C to retain the NLC fraction while allowing the aqueous fraction containing free caffeine to pass through the membrane. The recovered filtrate was diluted with methanol to a final volume of 10 mL. Free caffeine was quantified by UV spectrophotometry at 273 nm using the previously prepared calibration curve, and the EE% was subsequently obtained from the corresponding equation:

$$\% \text{ EE} = \frac{\text{Amount of drug added} - \text{Amount of free drug}}{\text{Amount of drug added}} \times 100$$

Differential Scanning Calorimetry (DSC) analysis

DSC was used to characterize the thermal properties and physical state of tripalmitin, pure caffeine, lyophilized blank NLCs, and lyophilized caffeine-loaded NLCs. Approximately 3 mg of each sample was weighed accurately and transferred into standard aluminum pans. Following sealing, a small hole was introduced into the center of each lid to permit pressure equilibration. An empty aluminum pan was used as the reference. Thermograms were obtained between 0 and 200°C at a heating rate of 5°C/min under a dynamic nitrogen atmosphere maintained at a flow rate of 50 mL/min using a DSC system operated with STARe software.

Drug-excipient compatibility study using Fourier-transform infrared spectroscopy (FT-IR)

Fourier-transform infrared spectroscopy was employed to assess possible chemical interactions between caffeine and the excipients constituting the lipid matrix. Spectra were obtained for pure caffeine, tripalmitin, lyophilized blank NLCs, and lyophilized caffeine-loaded NLCs using the KBr pellet method.

For pellet preparation, approximately 10 mg of each dried sample was thoroughly blended with spectroscopic-grade KBr at a 1:50 sample-to-KBr ratio using a mortar and pestle. The resulting homogeneous powder was transferred into a die and compressed with a hydraulic press at 10 tons for 2 min to form a translucent pellet. The prepared pellets were then positioned in the sample holder, and infrared spectra were collected using the instrument software (Spectrum). The spectra were examined for changes in characteristic absorption bands, including peak shifts or the emergence of additional bands that could indicate potential chemical incompatibility.

Preparation of HA-based gel and final formulation

A 1% (w/v) HA gel was prepared using a combination of high-molecular-weight (HMW) and low-molecular-weight (LMW) HA to provide the desired hydration and topical delivery characteristics. The optimized caffeine-loaded NLC dispersion was subsequently incorporated into the gel base at a 1:2 ratio under continuous stirring until a uniform formulation was obtained. Following incorporation, particle size, PDI, and zeta potential were reassessed to determine whether incorporation into the HA matrix altered the physicochemical characteristics of the NLCs.

Morphology analysis

The morphology of the NLCs before and after incorporation into the HA gel was examined by field-emission scanning electron microscopy (FE-SEM). For imaging, the NLC dispersion was diluted approximately 100-fold with distilled water. A 20 µL portion of the diluted suspension was deposited onto a carbon-coated grid and allowed to stand for 30 s. Excess liquid was removed carefully with filter paper, after which the specimens were air-dried and examined by FE-SEM.

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pH measurement

The pH of the final formulation was measured at room temperature using a calibrated pH meter after preparing a 10% (w/v) aqueous dispersion of the formulation.

Viscosity measurement

The viscosity of the final formulation was determined using a rotational viscometer equipped with a Cup and Bob geometry under controlled measurement conditions.

Ex vivo skin permeation study

The animal study protocol received approval from the Institutional Animal Ethics Committee (IR.MUMS.AEC.1404.009). Male BALB/c mice (25–30 g) were housed under standardized conditions consisting of a 12 h light/12 h dark cycle, with food and water available ad libitum. Before the experimental procedures, the animals underwent a 7-day acclimatization period. Experimental handling and procedures were carried out in accordance with IACUC principles, with measures implemented throughout the study to reduce unnecessary discomfort and distress.

The ex vivo distribution of caffeine across mouse skin was investigated using Franz diffusion cells containing excised dorsal skin. Three test preparations were included in the comparison: a conventional caffeine formulation containing HA and the same lipid and surfactant components as the NLC system but without high-energy processing; the caffeine-loaded NLC dispersion; and the optimized caffeine-loaded NLC incorporated into the HA gel.

Following euthanasia by CO_2 asphyxiation, the dorsal region of each mouse was first cleared of hair with an electric clipper and razor. The skin was then excised and rinsed with normal saline to remove any remaining tissue. Each skin specimen was subsequently mounted between the donor and receptor chambers of a Franz diffusion cell, taking care to maintain the correct orientation and ensure a leak-free assembly. PBS was used as the receptor medium, and its temperature was maintained at 32 ± 0.5°C through a circulating water jacket. The receptor medium was continuously agitated at 500 rpm.

For each experiment, 1 g of the corresponding formulation was introduced into the donor chamber. Aliquots were withdrawn from the receptor phase at 5, 10, 15, 20, and 30 min and at 2, 4, 6, 8, 12, 24, and 48 h. An equivalent volume of fresh PBS was added immediately after each sampling point to maintain sink conditions. The caffeine concentration in the collected receptor samples was measured at 273 nm by UV spectrophotometry using a pre-validated calibration curve.

The cumulative caffeine recovered from the receptor phase after 48 h was used to calculate the permeated fraction relative to the initial applied dose. Following completion of the experiment, caffeine remaining in the excised skin was extracted and quantified to determine the retained fraction, also expressed relative to the applied dose. A mass-balance analysis was additionally performed to assess the overall recovery of the administered caffeine.

Stability studies

Stability assessment was performed according to the ICH Q1A (R2) guideline (International Council for Harmonization, 2003). The optimized formulations were maintained under three storage conditions: 4 ± 2°C, 25 ± 2°C/60% RH, and 40 ± 2°C/75% RH, representing refrigerated, long-term, and accelerated conditions, respectively. Before sealing, the containers were flushed with nitrogen to reduce the possibility of oxidative degradation during storage, particularly given the lipidic nature of the formulations.

Samples were evaluated at 0, 1, 3, and 6 months for changes in mean particle size, PDI, zeta potential, and encapsulation efficiency. Refrigerated storage was additionally included instead of an intermediate condition to specifically assess the behavior of the lipid-based nanocarrier under low-temperature storage.

Statistical analysis

All measurements were obtained from three independent experimental replicates, and the data are expressed as mean ± standard deviation (SD). Statistical calculations were carried out with GraphPad Prism 9.0.

Differences among multiple groups were examined using one-way ANOVA with Tukey's post hoc multiple-comparison test. For experiments involving repeated time-dependent measurements, such as release and permeation studies, a two-way ANOVA was performed to evaluate the effects of formulation and time. Data normality was assessed before statistical testing. Statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Optimization and preparation of caffeine-loaded NLCs

Given the hydrophilic nature of caffeine and its limited affinity for lipid matrices, a preliminary qualitative solubility screening was conducted to

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identify suitable formulation components. Based on these results, cetyl palmitate (CP) and tripalmitin (TP) were selected as candidate solid lipids, while MCT oil was chosen as the liquid lipid to reduce matrix crystallinity and enhance drug accommodation. Tween 80 (HLB ≈ 15) was selected as the surfactant due to its appropriate hydrophilic–lipophilic balance for stabilizing systems containing hydrophilic compounds. MCT oil and Tween® 80 were therefore kept constant in subsequent formulations.

Preliminary NLCs were prepared using both CP and TP to evaluate their suitability. However, the CP-based formulation exhibited rapid gelation, indicating poor physical and colloidal stability, possibly due to polymorphic transitions or incompatibility within the lipid–surfactant system. In contrast, TP-based formulations showed stable dispersions with acceptable physicochemical properties and were therefore selected for further optimization (Table 1).

To investigate the effect of drug loading, TP-based NLCs were prepared with varying caffeine concentrations (0.1–3%). The influence of caffeine concentration on particle size, PDI, zeta potential, and encapsulation efficiency (EE %) was systematically evaluated (Table 2).

At lower concentrations (0.1–0.25%), the formulations exhibited smaller particle sizes and low PDI values, indicating relatively homogeneous systems. The highest EE% was observed at 0.1% caffeine (96.3 ± 0.7%); however, this is likely attributable to the low initial drug content rather than an inherently superior loading capacity. As caffeine concentration increased, EE% gradually decreased, reaching 56.5 ± 0.4% at 3%, which can be explained by the limited accommodation capacity of the lipid matrix and increased partitioning of excess drug into the aqueous phase.

Particle size remained relatively consistent up to 1% caffeine (~245 nm), but increased markedly at 3% (366.8 ± 8.3 nm), accompanied by a significant rise in PDI (0.366 ± 0.029), indicating reduced homogeneity and possible aggregation at higher drug loading. Zeta potential values did not follow a clear trend, suggesting that steric stabilization by Tween 80 likely played a more dominant role than electrostatic stabilization in maintaining dispersion stability.

Based on the overall evaluation, the formulation containing 1% caffeine was selected as the optimized system. Although lower concentrations demonstrated higher EE%, the 1% formulation provided a more appropriate balance between EE%, particle size, and dispersion homogeneity. This balance is particularly important for topical applications, where sufficient drug payload alongside acceptable physicochemical stability is required [18].

The optimized formulation, composed of TP (5.3%), MCT oil (1.5%), Tween 80 (5%), and caffeine (1%), exhibited a particle size of 240.5 ± 4.7 nm and an EE% of 77.1 ± 0.6%. The relatively high encapsulation efficiency can be attributed to the structural characteristics of NLCs, where incorporation of liquid lipids into the solid matrix creates imperfections that reduce drug expulsion and improve drug accommodation. These findings are consistent with previous reports indicating that NLC systems can enhance the loading of hydrophilic compounds through their less ordered internal structure [19].

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Table 1. Particle size, PDI, and zeta potential of preliminary caffeine-loaded NLCs prepared with different solid lipids

Formulation	Solid lipid	Particle size (nm)	PDI	Zeta potential (mV)
NLC-CP	Cetyl Palmitate 3.5%	1082.0 ± 28.9	0.907 ± 0.029	-23.0 ± 2.6
NLC-TP	Tripalmitin 3.5%	245.1 ± 5.5	0.134 ± 0.012	-19.9 ± 1.7

All formulations contained 1.5% MCT oil and 5% Tween® 80. Distilled water was added to a final volume of 10 mL.

Table 2. Effect of caffeine concentration on particle size, PDI, zeta potential, and encapsulation efficiency (EE %) of TP-based NLCs

	Caffeine (%)	Particle size (nm)	PDI	Zeta potential (mV)	EE%
NLC-0.1	0.1	147.5 ± 3.6	0.138 ± 0.015	-2.74 ± 1.8	96.3 ± 0.7
NLC-0.2	0.2	245.1 ± 5.5	0.134 ± 0.012	-19.90 ± 1.7	89.4 ± 0.3
NLC-0.25	0.25	240.3 ± 6.4	0.082 ± 0.022	-3.21 ± 2.1	87.9 ± 0.5
NLC-0.5	0.5	213.6 ± 7.9	0.098 ± 0.028	-10.50 ± 0.9	80.2 ± 0.6
NLC-1	1	240.5 ± 4.7	0.177 ± 0.020	-8.09 ± 1.3	77.1 ± 0.6
NLC-3	3	366.8 ± 8.3	0.366 ± 0.029	-3.24 ± 2.6	56.5 ± 0.4

All formulations contained 3.5% tripalmitin, 1.5% MCT oil, and 5% Tween® 80. Distilled water was added to a final volume of 10 mL.

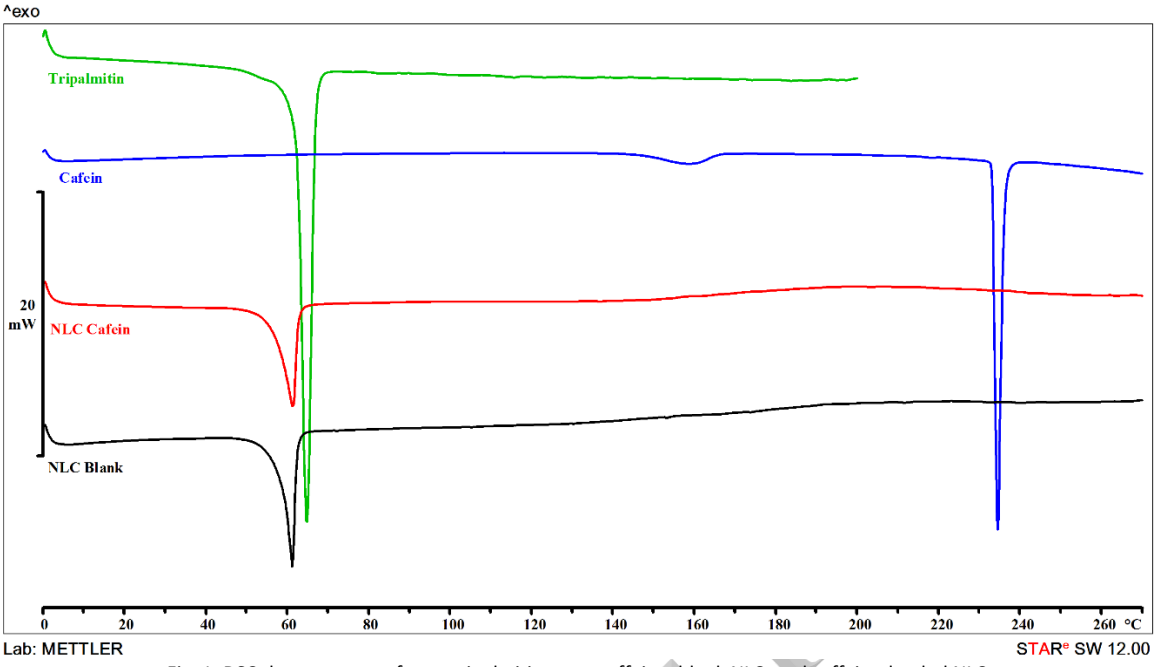

Fig. 1. DSC thermograms of pure tripalmitin, pure caffeine, blank NLC, and caffeine-loaded NLC.

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DSC analysis

The thermal characteristics and physical state of caffeine incorporated within the lipid matrix were evaluated by DSC. As presented in Figure 1, the thermogram of pure caffeine displayed a distinct endothermic event at approximately 233–235 °C, which corresponds to its melting transition and confirms the crystalline nature of the unprocessed drug [20].

In contrast, this characteristic peak was absent in the thermogram of caffeine-loaded NLCs. This observation suggests that caffeine was no longer present in its crystalline form but was either molecularly dispersed or present in an amorphous state within the lipid matrix. Such behavior is commonly associated with successful incorporation of the drug into lipid-based nanocarriers [21].

Furthermore, the thermogram of the blank NLC displayed a broadened and slightly shifted lipid melting peak compared to the pure solid lipid (TP), indicating reduced crystallinity of the lipid matrix. This reduction can be attributed to the presence of the liquid lipid (MCT oil), which introduces structural imperfections into the lipid network [22].

Although the absence of a distinct caffeine melting peak supports its incorporation into the NLC system, it does not necessarily confirm complete encapsulation. However, this finding, in combination with the relatively high encapsulation efficiency (77.1 ± 0.6%), suggests that a significant fraction of caffeine was successfully accommodated within the lipid matrix [23].

Drug-excipient compatibility study using FT-IR

Potential interactions between caffeine and the formulation components were examined by FT-IR spectroscopy. The spectrum obtained for pure caffeine exhibited the characteristic vibrational bands of the molecule, including those attributed to C=O stretching at approximately 1700 cm^{-1}, C–N stretching, and N–H bending [24].

As illustrated in Figure 2, these characteristic peaks were retained in the spectrum of the caffeine-loaded NLC, with no significant shifts or disappearance of major bands. This indicates that caffeine's chemical structure remained unchanged during formulation and that no strong chemical interactions or bond formation occurred between the drug and lipid components.

The spectra of blank NLC and tripalmitin showed typical lipid-associated bands, including C–H stretching vibrations in the range of 2800–2900 cm^{-1} and ester carbonyl peaks around ~1735 cm^{-1}. These peaks were also present in the caffeine-loaded formulation, confirming the integrity of the lipid matrix [25].

Overall, the FT-IR results suggest that caffeine is physically incorporated within the lipid matrix without undergoing chemical modification, supporting the compatibility of the drug with the selected excipients.

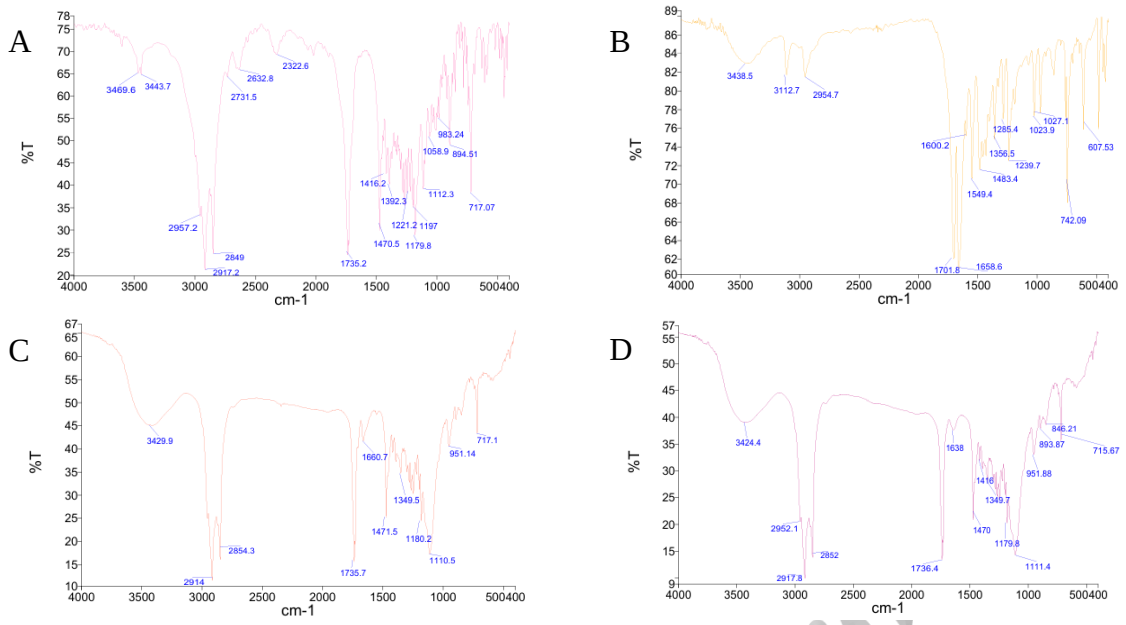

Fig. 2. FT-IR spectra of (A) tripalmitin, (B) pure caffeine, (C) caffeine-loaded NLC, and (D) blank NLC in the range of 4000–400 cm^{-1}.

Preparation of HA-based gel and final formulation

For development of the final topical formulation, a 1% (w/v) HA gel containing both high-molecular-weight (HMW) and low-molecular-weight (LMW) hyaluronic acid was first prepared. The optimized caffeine-loaded NLC dispersion was then incorporated into the gel base at a 1:2 ratio, yielding the final caffeine-NLC-HA formulation.

Following preparation, the impact of the gel matrix on nanoparticle characteristics was evaluated. As presented, the particle size distribution, PDI, and zeta potential of the final formulation were 242.5 ± 4.2 nm, 0.322 ± 0.022, and −16.7 ± 0.8 mV, respectively. These results indicate that incorporation of NLCs into the viscous HA gel did not adversely affect the colloidal properties.

The maintenance of the Z-average and PDI within the optimal range suggests that the structural integrity and physical stability of the caffeine-loaded nanoparticles were preserved within the gel network.

Morphology analysis

FE-SEM was employed to evaluate the surface morphology, particle shape, and potential aggregation of the plain caffeine-loaded NLC dispersion and the caffeine-loaded NLCs incorporated into the HA gel. As illustrated in Figures 3 and 4, the micrographs revealed discrete nanoparticles with a relatively uniform and spherical morphology. The observed particle size was approximately 200 nm for both formulations, which was generally consistent with the hydrodynamic particle size obtained by particle size analysis (PSA).

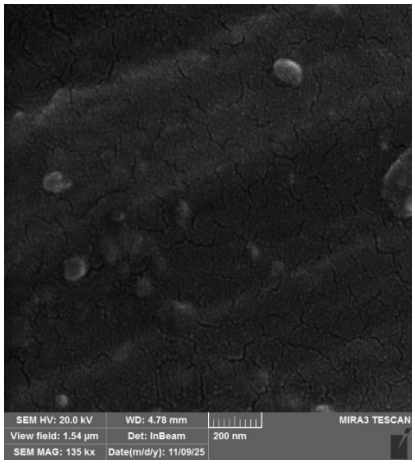

Fig. 3. FE-SEM images of optimized caffeine-loaded NLC formulation without HA gel.

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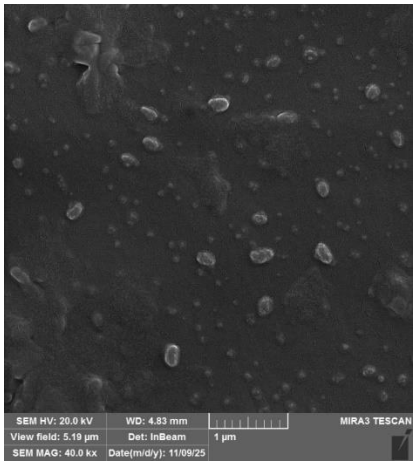

Fig. 4. FE-SEM images of optimized caffeine-loaded NLC formulation within HA gel.

The FE-SEM micrographs further demonstrated a relatively homogeneous distribution of the nanoparticles without apparent aggregation. The preservation of the spherical morphology and the absence of pronounced agglomeration after incorporation into the HA gel suggest that the overall external morphology of the NLCs was maintained within the gel matrix. These findings also indicate that the incorporation process did not result in an obvious loss of the characteristic particle morphology observed for the NLC dispersion.

The relatively uniform morphology observed in both formulations is consistent with the physicochemical characteristics expected for NLC-based systems and may contribute to their favorable formulation properties. However, particle morphology alone does not establish enhanced skin penetration. The effects of the NLC and HA gel components on caffeine delivery are therefore discussed in conjunction with the particle-size characteristics, drug-release behavior, and ex vivo skin permeation and retention findings presented in the subsequent sections [26].

pH determination

The pH value of the final formulation was determined using a 10% (w/v) aqueous dispersion. The measured pH at 25 ± 1°C was 6.4 ± 0.12. This value falls within the physiological range of human skin (approximately 4.5–6.5), indicating good cutaneous compatibility [27].

Accordingly, the formulation is expected to present a minimal risk of irritation or sensitization upon topical application. Maintaining a pH close to the natural acidity of the skin is essential for preserving the balance of the cutaneous microbiota and supporting skin barrier function [27].

This property is particularly important for topical formulations intended for application on sensitive regions, such as the periocular area, where the skin is thinner and more susceptible to irritation.

Viscosity measurement

The rheological behavior of the final formulation (caffeine-NLCs incorporated into HA gel) was investigated using a rotational viscometer with Cup and Bob geometry. As illustrated in Figure 5, an increase in shear rate resulted in a corresponding decrease in viscosity, while shear stress exhibited an increasing trend. This profile is characteristic of a non-Newtonian fluid with pseudoplastic (shear-thinning) behavior.

In such systems, the internal microstructure (polymer chains and/or dispersed particles) remains more entangled at rest or under low shear conditions, resulting in higher resistance to flow. However, with increasing shear rate, these structures progressively align and disentangle in the direction of flow, leading to a reduction in viscosity.

This rheological behavior is highly advantageous for semi-solid topical formulations, as it facilitates easy spreadability during application while maintaining adequate structural integrity and preventing phase separation during storage [28].

Furthermore, the rheogram presented in Figure 6 indicates thixotropic behavior. In this context, viscosity decreases under shear and gradually recovers upon removal of the applied force. This time-dependent reversible behavior is attributed to the breakdown of the internal three-dimensional network under shear and its subsequent reconstruction during the resting state.

The presence of thixotropy is particularly desirable for periocular applications, as it allows sufficient fluidity for uniform distribution without excessive dragging of the delicate skin, while also preventing post-application runoff due to viscosity recovery [28].

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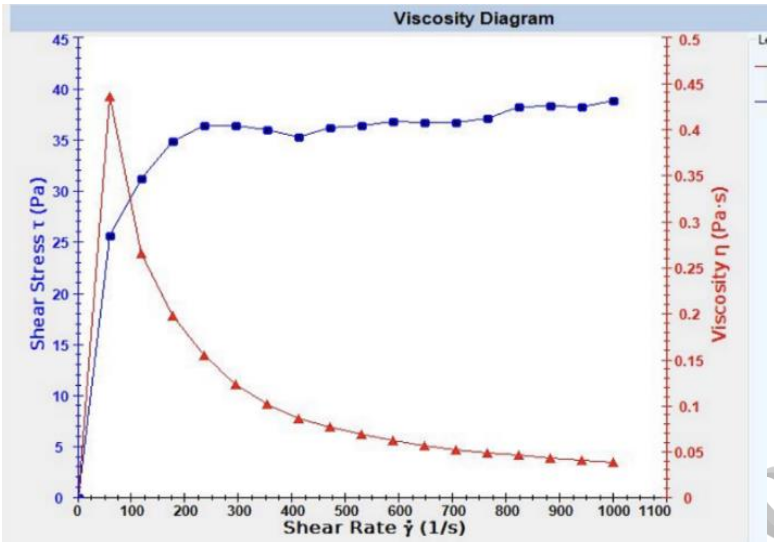

Fig. 5. Viscosity profile of the final formulation (caffeine-loaded NLCs incorporated into HA gel) measured using a Cup and Bob rotational viscometer.

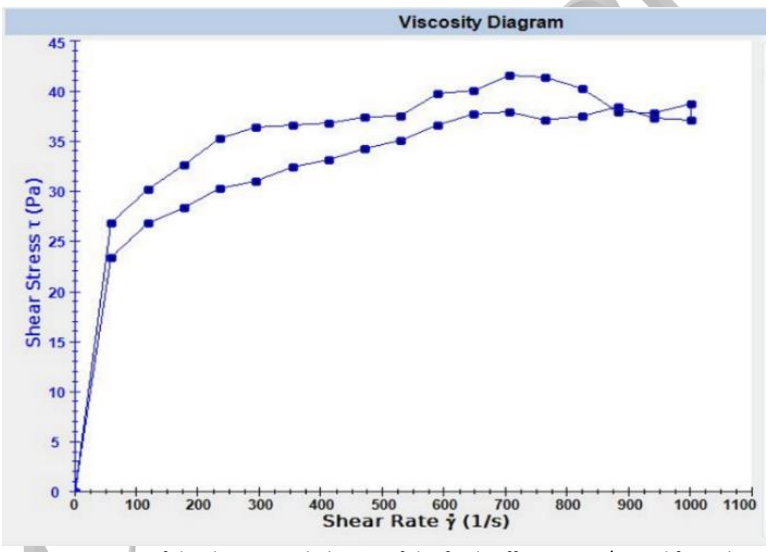

Fig. 6. Assessment of the thixotropic behavior of the final caffeine-NLCs/HA gel formulation.

Overall, these rheological properties contribute to improved application performance, favorable sensory attributes, and enhanced physical stability of the NLC-based gel system.

Ex vivo skin permeation study

As previously described, the skin penetration and retention profiles of caffeine from NLC dispersions (with and without HA) and the conventional formulation were evaluated using Franz diffusion cells. The corresponding penetration and retention percentages are presented in Table 3. Statistical analysis was performed using GraphPad Prism 9 (one-way ANOVA followed by Tukey's multiple comparisons test) to assess the significance of differences among the study groups over the 48 hours. Although time-dependent profiles were recorded, only endpoint analysis at 48 h was used for statistical comparison.

Comparison of the three formulations demonstrated a pronounced formulation-dependent distribution of caffeine between the skin and receptor compartment (Table 3). Caffeine-loaded NLCs without HA showed lower skin permeation (26.79 ± 0.0019%) and higher skin retention (71.24 ± 0.002%) than the conventional formulation, which exhibited 91.01 ± 0.02% permeation and only 7.03 ± 1.02% retention ($p < 0.05$). The reduced transdermal transport observed with the NLC formulation may be related to incorporation of caffeine within the lipid-based nanostructured matrix, which can provide a reservoir-like microenvironment and modify drug availability and release at the skin interface. Previous studies have demonstrated that the carrier system can substantially influence the penetration and permeation behavior of caffeine and other topically applied drugs [29, 30].

Table 3. The skin penetration and retention percentages of formulations

Formulation	Skin penetration (%)	Skin retention (%)
Conventional formulation	91.01 ± 0.02	7.03 ± 1.02
Caffeine-NLC without HA	26.7900 ± 0.0019	71.240 ± 0.002
Caffeine-NLC + HA	4.580 ± 0.002	94.250 ± 0.003

Table 4. Physicochemical stability of optimized caffeine-loaded NLCs under different storage conditions over 6 months

Time (month)	Conditions	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)
1	Refrigerated	236 ± 4.2	0.177 ± 0.011	-7.11 ± 1.1	76.3 ± 1.2
	Long-term	224.9 ± 3.6	0.177 ± 0.021	-3.93 ± 0.7	
	Accelerated	229.9 ± 5.1	0.152 ± 0.027	-6.75 ± 1.5	
3	Refrigerated	213.7 ± 5.8	0.121 ± 0.023	-10.01 ± 0.9	75.8 ± 0.5
	Long-term	211.8 ± 8.2	0.137 ± 0.028	-9.07 ± 1.1	
	Accelerated	207.6 ± 4.3	0.140 ± 0.019	-6.71 ± 2.3	
6	Refrigerated	210.4 ± 2.6	0.136 ± 0.019	-9.14 ± 1.3	74.9 ± 0.8
	Long-term	221.4 ± 7.3	0.125 ± 0.021	-7.13 ± 2.1	
	Accelerated	219.6 ± 3.9	0.134 ± 0.026	-6.64 ± 1.9	

Caffeine is a hydrophilic molecule, and its skin penetration is therefore strongly influenced by the formulation and delivery system. In the NLC formulation, association of caffeine with the lipid matrix may limit its rapid diffusion toward the receptor compartment and favor its localization within the skin. Similar effects of lipid nanocarriers on local skin deposition and retention have been reported for other topical drugs [23]. Importantly, this interpretation does not imply that NLCs bypass the stratum corneum; rather, the present findings suggest that the NLC system modifies caffeine availability and distribution, resulting in greater dermal retention relative to transdermal transport.

The incorporation of HA further shifted caffeine distribution toward the skin, with the NLC–HA formulation showing markedly higher retention (94.25 ± 0.003%) and lower permeation (4.58 ± 0.002%). HA should therefore not be considered merely as a thickening agent. Owing to its hydrophilic and bioadhesive properties, HA can retain water at the skin surface, increase formulation–skin contact, and modify the hydration state of the stratum corneum [31]. Such effects may influence the delivery of hydrophilic compounds; however, their final effect depends on the delivery system in which the drug is incorporated. This is evident in the present study, where HA-containing conventional formulation showed high permeation and low retention, whereas incorporation of the same drug into NLCs produced the opposite distribution pattern.

Thus, the enhanced skin retention observed with the NLC–HA formulation is more appropriately interpreted as a complementary effect of the two components rather than as direct enhancement of NLC penetration by HA. HA may provide a hydrated and adhesive environment that prolongs formulation residence at the skin surface, while the NLC lipid matrix controls caffeine availability and favors its localization within the skin [32]. Overall, the marked increase in skin retention to 94.25 ± 0.003%, together with the reduction in receptor-phase permeation to 4.58 ± 0.002%, supports the potential of the NLC–HA system for localized topical caffeine delivery while limiting transdermal transport.

Stability studies

Particle size, zeta potential, and PDI are critical quality attributes of nanoparticulate systems, directly influencing physical stability, drug release behavior, and biological performance. In particular, the hydrodynamic diameter (Z-average) plays a key role in determining the colloidal stability and in vivo fate of nanocarriers.

As shown in Table 4, no pronounced instability or aggregation was observed across the three storage conditions (4°C, 25 ± 2°C with 60% RH, and 40 ± 2°C with 75% RH) over the studied period. Overall, particle size variations remained within a narrow range, suggesting acceptable physical stability of the optimized formulation under different environmental stresses.

The PDI values remained consistently low (generally < 0.2), indicating a homogeneous and narrowly distributed nanoparticle population. Since PDI values above 0.5 are typically associated with broad size distribution and potential instability, the obtained results confirm the colloidal uniformity of the system throughout the storage period.

Interestingly, a slight reduction in particle size was observed at later time points compared to baseline ($p < 0.05$). This behavior may be attributed to lipid matrix reorganization and polymorphic transitions within triglyceride-based solid lipids, which can occur during storage. Such structural rearrangements have previously been

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reported to lead to a more compact lipid packing and consequently a reduction in apparent particle diameter [25].

Regarding zeta potential, the relatively low negative values observed across all conditions suggest limited electrostatic stabilization; however, the stability of the system can be attributed mainly to steric stabilization provided by the surfactant layer rather than electrostatic repulsion [33].

EE% remained relatively stable over the storage period, with only minor fluctuations, indicating that drug leakage from the lipid matrix was minimal. This further supports the robustness of the formulation under both refrigerated and accelerated conditions.

Overall, the stability profile demonstrates that the optimized caffeine-loaded NLC system maintains its physicochemical integrity over time, confirming its suitability as a stable nanocarrier for topical delivery applications.

CONCLUSION

Based on the findings of this investigation, caffeine-loaded NLCs were successfully developed and optimized. The physicochemical evaluations demonstrated that the selected formulation possessed an appropriate particle size, low PDI, and a relatively stable negative zeta potential, indicating acceptable colloidal stability and homogeneity of the system. Furthermore, the relatively high encapsulation efficiency confirmed the suitability of the selected lipid matrix and solid-to-liquid lipid ratio for effective caffeine incorporation.

Characterization studies also provided important information regarding the structural characteristics of the developed NLCs. The DSC results suggested that caffeine was largely amorphous or molecularly dispersed within the lipid matrix. Consistent with this finding, FT-IR analysis revealed no notable chemical interactions between caffeine and the formulation excipients. Furthermore, FE-SEM images showed predominantly spherical nanoparticles with a relatively homogeneous distribution.

In terms of performance, the ex vivo permeation study demonstrated a clear formulation-dependent behavior. The caffeine-loaded NLC system significantly enhanced skin retention while reducing transdermal permeation compared to the conventional formulation. Moreover, incorporation into a HA-based gel further improved dermal deposition, suggesting a synergistic effect between the lipid nanocarrier and the hydrophilic polymeric network.

A pivotal finding of this study was the dual role of the HA gel depending on the delivery system. When incorporated into the NLC formulation, HA contributed to increased skin hydration and enhanced localization of the nanoparticles within the superficial skin layers, thereby maximizing local retention. In contrast, in the conventional formulation, HA appeared to facilitate drug permeation across the skin barrier, leading to increased systemic transport.

The optimized formulation also exhibited good stability over three months when stored under refrigerated (4 ± 2°C), long-term (25 ± 2°C, 60% RH), and accelerated (40 ± 2°C, 75% RH) conditions. No statistically significant differences were detected in particle size, PDI, or zeta potential during the study period, indicating satisfactory physicochemical stability and overall formulation robustness.

In conclusion, the developed caffeine-loaded NLC system, particularly when incorporated into an HA gel, represents a promising, stable, and effective strategy for topical delivery, especially for application in delicate periocular skin regions. Compared to conventional formulations, this system offers superior performance in terms of controlled release behavior, enhanced localized skin deposition, and reduced systemic permeation of caffeine.

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AUTHOR'S CONTRIBUTIONS

Sh.G.: conceptualization, supervision, methodology, visualization, writing – review and editing; **S.S.:** Project administration, writing – original draft; **F.R.:** data curation, formal analysis, software, writing – review and editing.

CONFLICT OF INTEREST

The authors report no financial or personal conflicts of interest that could have affected the design, conduct, interpretation, or reporting of this study.

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ETHICAL CONSIDERATIONS

This study is approved in accordance with international guidelines and ethical research principles (code: IR.MUMS.AEC.1404.009).

AI STATEMENT

AI-assisted tools were used during manuscript preparation, primarily for language refinement and drafting support. The authors independently reviewed and edited all AI-assisted output and accept full responsibility for the content,

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accuracy, integrity, and originality of the final manuscript.

REFERENCES

1. Roh MR, Chung KY. Infraorbital dark circles: definition, causes, and treatment options. Dermatol Surg. 2009; 35(8): 1163–1171.
2. Sarkar R, Ranjan R, Garg S, Garg VK, Sonthalia S, Bansal S. Periorbital hyperpigmentation: a comprehensive review. J Clin Aesthet Dermatol. 2016; 9(1): 49.
3. Elias ML, Israeli AF, Madan R. Caffeine in skincare: Its Role in skin Cancer, sun protection, and cosmetics. Indian J Dermatol. 2023; 68(5): 546–550.
4. Herman A, Herman AP. Caffeine's mechanisms of action and its cosmetic use. Skin Pharmacol Physiol. 2012; 26(1): 8–14.
5. Brandner J, Behne M, Huesing B, Moll I. Caffeine improves barrier function in male skin. Int J Cosmet Sci. 2006; 28(5): 343–347.
6. Ahmadraji F, Shatalebi MA. Evaluation of the clinical efficacy and safety of an eye counter pad containing caffeine and vitamin K in emulsified Emu oil base. Adv Biomed Res. 2015; 4(1): 10.
7. Lin M, Qi X. Advances and challenges of stimuli-responsive nucleic acids delivery system in gene therapy. Pharmaceutics. 2023; 15(5): 1450.
8. Man M, Xin S, Song S, Cho S, Zhang X, Tu C, et al. Variation of skin surface pH, sebum content and stratum corneum hydration with age and gender in a large Chinese population. Skin Pharmacol Physiol. 2009; 22(4): 190–199.
9. Fouad SA, Badr TA, Abdelbary A, Fadel M, Abdelmonem R, Jasti BR, et al. New insight for enhanced topical targeting of caffeine for effective cellulite treatment: in vitro Characterization, permeation Studies, and histological evaluation in rats. AAPS Pharm Sci Tech. 2024; 25(7): 237.
10. Shiehzadeh F, Mir E, Raeesi S, Daneshmand S, Shahraki J. Nanostructured lipid carriers (NLCs) composed of argan oil, the potential novel vehicle for caffeine delivery to stratum corneum and hair follicles. Nanomed J. 2022; 9(1).
11. Ataee Nakhaei H, Rezaei F, Golmohammadzadeh S. Beyond conventional comparisons: a critical and integrative review of lipid-based and vesicular nanocarriers in cosmetic applications. Nanomed J. 2026: e28216.
12. Chylińska N, Maciejczyk M. Hyaluronic acid and skin: its role in aging and wound-healing processes. Gels. 2025; 11(4): 281.
13. Nam G, Lee HW, Jang J, Kim CH, Kim KH. Novel conformation of hyaluronic acid with improved cosmetic efficacy. J Cosmet Dermatol. 2023; 22(4): 1312–1320.
14. Zulqarnain M, Deepak P, Yarur AJ. Therapeutic drug monitoring in perianal fistulizing Crohn's disease. Journal Clin Med. 2022; 11(7): 1813.
15. Goudanavar P. Advancing topical drug delivery: NLC innovations in dermatological therepeutics. Nano Trends. 2025; 10: 100108.
16. D'Arcangelis A, Goswami Chatterjee S, Diaz I, Guehenneux S, Namkoong J, Wu J. In vitro, ex vivo, instrumental and clinical evaluation of a topical cream on the signs of periorbital ageing. Int J Cosmet Sci. 2025; 47(1): 18–30.
17. Ataee Nakhaei H, Rezaei F, Mahmoudi A, Tayarani-Najaran Z, Golmohammadzadeh S. Preparation and characterization of diosmin-loaded nanostructured lipid carriers for topical drug delivery and investigation of their antioxidant effects on the B16F10 melanoma cell line. J Pharm Innov. 2026; 21(3): 226.
18. Kassem AA, Asfour MH, Abd El-Alim SH, Khattab MA, Salama A. Topical caffeine-loaded nanostructured lipid carriers for enhanced treatment of cellulite: A 32 full factorial design optimization and in vivo evaluation in rats. Int J Pharm. 2023; 643: 123271.
19. Silva AC, Loureiro JA. A simple method for the preparation and characterization of nanostructured lipid carriers (NLC) formulations for cutaneous use. J Chem Educ. 2024; 101(10): 4333–4338.
20. Becerra-Lovera AY, Anaya-Mancipe JM, Díaz-Martin RD, Dias ML, Souza DdHS. Eugenol-Based Epoxy Vitrimers: Caffeine and Zinc Acetate as Potential Alternative Catalysts in Curing Kinetics and Dynamic Network Properties. Molecules. 2026; 31(5): 783.
21. Kim B-S, Na Y-G, Choi J-H, Kim I, Lee E, Kim S-Y, et al. The improvement of skin whitening of phenylethyl resorcinol by nanostructured lipid carriers. Nanomaterials. 2017; 7(9): 241.
22. Bhalekar MR, Pokharkar V, Madgulkar A, Patil N, Patil N. Preparation and evaluation of miconazole nitrate-loaded solid lipid nanoparticles for topical delivery. AAPS PharmSciTech. 2009; 10(1): 289–296.
23. Hoseinsalari A, Atapour-Mashhad H, Asili J, Tayarani-Najaran Z, Mohtashami L, Khanavi M, et al. Preparation and characterization of nanostructured lipid carriers (NLCs) containing glycyrrhiza glabra extract for the treatment of skin hyperpigmentation. Curr Pharm Des. 2024; 30(40): 3213–3232.
24. Elvis NI, Obinna OP, Chikeobi OF, Jennifer SM. Comparative FTIR Characterization of Caffeine Functional Groups in Different Varieties of Kola Nuts (Cola Acuminata and Cola Nitida). Int J Res Innov Appl Sci. 2026; 11(2).
25. Atapour-Mashhad H, Tayarani-Najaran Z, Golmohammadzadeh S. Preparation and characterization of novel nanostructured lipid carriers (NLC) and solid lipid nanoparticles (SLN) containing coenzyme Q10 as potent antioxidants and antityrosinase agents. Heliyon. 2024; 10(11).
26. Niu J, Yuan M, Wang L, Zhang P, Cui X, Fan Y, et al. Keratin-functionalized transfersomes for improving the skin permeation and deposition of podophyllotoxin. ACS Omega. 2025; 10(35): 40422–40434.
27. Brooks SG, Mahmoud RH, Lin RR, Fluhr JW, Yosipovitch G. The skin acid mantle: an update on skin pH. J Invest Dermatol. 2025; 145(3): 509–521.
28. Kundu S, Callahan K, Murthy SN. Rheological behavior of topical pharmaceutical formulations. Topical

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Products and Dermal Drug Delivery: Springer. 2025; 27–46.
29. Abd E, Roberts MS, Grice JE. A comparison of the penetration and permeation of caffeine into and through human epidermis after application in various vesicle formulations. Skin Pharmacol Physiol. 2016; 29(1): 24–30.
30. Luo L, Lane ME. Topical and transdermal delivery of caffeine. Int J Pharm. 2015; 490(1-2): 155–164.
31. Jacob S, Nair AB. Nanoemulgels as Advanced Topical Drug Delivery Systems: Mechanistic Insights and Therapeutic Applications in Skin Disorders, Infections, Wound Healing, and Cancer. Pharmaceuticals. 2026; 19(2): 247.
32. Chauhan N, Kumar A, Jyoti A, Shah P, Desai N, Lalan M. Hyaluronic Acid-functionalized Hesperidin-loaded Solid Lipid Nanoparticles for Mitigating Oxidative Stress: A Potential Strategy for Radiation-induced Skin Injury. Appl Biochem Biotechnol. 2026: 1–28.
33. Anjum R, Lakshmi P. A review on solid lipid nanoparticles; focus on excipients and formulation techniques. Int J Pharm Sci Res. 2019; 10(9): 4090.

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