

ORIGINAL ARTICLE

Enhanced Topical Delivery of Rosehip Oil (RO) through Nanostructured Lipid Carriers (NLC)

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Abstract

Introduction: Rosehip oil (RO) is rich in bioactive constituents with potential for various formulations. This investigation aims to design and analyze Nanostructured Lipid Carriers (NLC) containing RO to protect its delicate components, prolong RO's shelf life, and enhance its permeability.

Methods: The NLC formulation was created using high shear homogenization, with RO as the liquid lipid and Compritol 888 ATO (C888) as the solid lipid. A series of assessments was conducted to evaluate encapsulation effectiveness, particle size distribution, polydispersity index, zeta potential, ex vivo skin penetration, and stability.

Results: The NLCs showed uniform spherical morphology, average particle sizes of 180 nm, PDI below 0.3, and zeta potential around -30 mV. High encapsulation efficiency was also observed. Ex vivo experiments indicated improved Quercetin retention in both Stratum Corneum (SC) and viable epidermis/dermis layers with NLC-encapsulated RO compared to RO alone. Stability assessments showed that the NLC formulation remained stable at 4°C for at least 90 days.

Discussion and Conclusion: The findings support NLC's efficacy in delivering active compounds to the skin, presenting an encouraging alternative for treating skin conditions.

Keywords: Nanoparticles; nanostructured lipid carriers; quercetin; rosehip oil; skin permeation.

The production of reactive oxygen species (ROS) is a natural byproduct of cellular metabolic processes, with mitochondria being the primary source. However, when ROS generation surpasses the body's antioxidant capability, it can lead to damage in vital macromolecules like lipids, proteins, and DNA^[1,2]. This excess ROS production, particularly in the skin, is increasingly acknowledged as a crucial element in the emergence of various cutaneous pathologies, including some forms of skin cancer^[3,4]. These ROS are also implicated in a variety of

pathologies, including immunosuppression, photoaging, and photocarcinogenesis^[5,6]. The use of antioxidants therapeutically as a means to counteract these pathologies has shown significant promise^[7,8].

Originating from the Rosa genus in the Rosaceae family, rosehip oil (RO) is a type of fruit that boasts an abundant array of indispensable compounds^[9,10]. Their distinct characteristics have earned them a standing for demonstrating a broad variety of health advantages, which include antioxidant, antimicrobial, anti-inflammatory,

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antidiabetic, and even anticancer impacts^[11,12]. Due to the presence of various compounds, they become susceptible to influences like oxygen exposure, light, UV irradiation, and acidity level shifts^[13,14]. Additionally, their limited absorption limits their capability to deliver the intended therapeutic outcomes. To circumvent these limitations, the fabrication of specialized carriers for RO becomes imperative. These carriers are tasked with safeguarding the integrity of the active substances and ensuring their sustained release, facilitating more convenient oral or dermal applications^[15,16].

In recent years, nanocarriers have emerged as a global focal point in pharmaceutical and cosmetic sciences, with nanostructured lipid carriers (NLC) standing out as a particularly promising advancement^[17–21]. NLC offer a host of advantageous attributes, ranging from heightened therapeutic efficacy to enhanced skin hydration and prolonged stability of encapsulated active ingredients^[22,23]. These carriers have been generated by uniting solid and liquid lipids, along with surfactants, leading to a formula that has extremely promising prospects for a broad range of uses^[24].

The goal of this research was to prepare an NLC incorporating RO for the first time, elevating its topical application. NLCs were made using a technique known as high shear homogenization, followed by a sonication process. Thorough assessments encompassing physicochemical properties and stability were carried out on the NLC formulation. Furthermore, *ex vivo* permeability examinations were undertaken to evaluate the capacity of NLC for dermal absorption.

Materials and Methods

Materials

Rosehip oil (RO) was obtained from Talya Herbal Company, Türkiye. Compritol 888 ATO (C888) was graciously supplied as a free sample by Gattefosse Company. Quercetin and Tween®80 (Tw80) were acquired from Sigma–Aldrich, Inc. All other chemicals employed in this research were of analytical-grade standards.

Preparation of NLC

The NLC containing RO was prepared following a modified high shear homogenization technique^[25,26]. Compritol 888 ATO (C888) was selected as the solid lipid component, while Rosehip oil (RO) was used as the liquid lipid phase. Tween 80 (Tw80) was employed as the surfactant to stabilize the formulation. To initiate the formulation process, the lipid

phase, comprising C888 and RO, was carefully combined and heated to 85°C. Simultaneously, the aqueous phase, consisting of Tw80 dissolved in distilled water, was also heated to the same temperature. Subsequently, the warm aqueous phase was slowly added to the heated oily phase while maintaining continuous agitation for a duration of 5 minutes at 20000rpm, facilitated by an Ultra-Turrax (T25, IKA, Germany). This ensured thorough mixing and uniform distribution of components. Following the emulsification step, the resulting pre-emulsion underwent sonication using a probe set at 40% amplitude for a duration of 10 minutes. This additional step was essential for further reducing the nanoparticle size, contributing to improved stability and uniformity. Subsequently, the NLC was cooled in an icy bath for a period of about 10 minutes. This modified method has been shown to effectively reduce nanoparticle size, thereby enhancing the overall stability and uniformity of the NLC formulations^[19]. The components of NLC preparations are described in Table 1.

Determination of Particle Size, Polydispersity, and Zeta Potential

The measurements of particle size and polydispersity index of NLC were taken using dynamic light scattering, with the Zetasizer Nano ZSP instrument (Malvern Instruments Ltd, Malvern, UK). The Zetasizer Nano ZSP was also used for measuring the zeta potential, utilizing custom cuvettes. To minimize the impact of multiple scattering, the NLCs were diluted with deionized water at a ratio of 1:50. The measurements were conducted in a room at a consistent temperature, and each analysis was repeated three times to ensure statistical reliability.

Encapsulation Efficiency

The encapsulation efficiencies of the NLCs were analyzed using a modified centrifugation technique, as described in the literature. The NLC dispersions were centrifuged at 20000g for 1 hour. Subsequently, the supernatant was carefully separated to remove any non-encapsulated drug and free surfactants. The isolated supernatant was then diluted with acetonitrile for subsequent quantification,

Table 1. The ingredients of NLC

Code	Solid lipid (mg)	Liquid lipid (mg)	Surfactant (%)	PBS pH 7.4 (mL)
F1	200	10	1	10
F2	200	10	3	10
F3	200	10	5	10

which was accomplished using a validated reversed-phase high-pressure liquid chromatography (RP-HPLC) method.

Quercetin was selected as the marker for the quantitative assessment of RO. Through RP-HPLC analysis, it was determined that unbound quercetin was present in the supernatant. The quantification of quercetin entrapment was calculated by subtracting the amount of free quercetin from the total initial quercetin. The encapsulation efficiency was calculated using this formula:

$$\text{Encapsulation Efficiency \%} = \frac{\text{the amount of total drug} - \text{the amount of non-encapsulated drug}}{\text{the amount of total drug}} * 100$$

The concentration of free Quercetin was assessed using an RP-HPLC system (model LC20-AT, Shimadzu, Kyoto, Japan) equipped with a UV-Vis detector. A GL Sciences InertSustain C18 column (5 μm particle size; 250 \times 4.6 mm) was utilized for the chromatographic separation. A 20 μL aliquot was injected into the system. The mobile phase consisted of a combination of 80% acetonitrile and 20% water containing 0.1% acetic acid. Analysis was conducted at a flow rate of 1.5 mL/min, with detection at a wavelength of 370 nm.

Ex-vivo Skin Permeation

The *ex-vivo* skin permeability investigation was undertaken to assess the impact of topical formulations on skin permeation^[27]. Ear skins from 6-month-old pigs were sourced from a local slaughterhouse. Dorsal skin sections were carefully excised from the cartilage using a scalpel, ensuring uniformity. Prior to experimentation, the skin surface was prepared by gently removing hair using surgical scissors, without compromising the stratum corneum (SC). Subcutaneous tissues were meticulously separated from the skin using a scalpel. The pig skins were refrigerated at -20°C until needed and permitted to reach equilibrium at ambient temperature prior to experimentation. To ensure proper hydration, the skins were immersed in phosphate-buffered saline. In this investigation, a customized version of the double-jacketed Franz diffusion cell, specifically the Hanson Vertical Diffusion Cell, featuring a receptor phase volume of 7 mL and a diffusion surface area of 1.77 cm², was utilized. The receptor phase consisted of a 1:1 mixture of ethanol and PBS (pH 7.4) to facilitate solubility of the active ingredients. To assess the efficacy of the carrier system, NLC formulations and RO containing equal amounts of quercetin were applied to the dermal layer. The diffusion apparatus was consistently maintained at a steady temperature of 37 \pm 0.5°C to mimic physiological conditions. At predetermined intervals, 0.5 mL samples

were withdrawn from the receptor phase, passed through a 0.45 μm syringe filter, and replaced with an equal volume of fresh solvent mixture to maintain the receptor phase volume. After 6 hours, the permeation study was concluded.

After the allocated sampling duration, skin specimens were meticulously retrieved from the apparatus, washed with PBS solution, and subsequently dried with cotton wool. The stratum corneum (SC) was then gently removed using adhesive tape strips (Corneofix). This process was repeated 20 times, exerting mild pressure while smoothly affixing the tape onto the skin's surface. Subsequently, both the tape strips and residual skin specimens underwent a 24-hour immersion in acetonitrile, followed by a series of agitation for extraction. Extracted aliquots were analyzed using a validated HPLC method to quantify the quercetin.

Stability of NLC

The study employed a stability assessment protocol to evaluate the selected NLC formulations. The formulations were stored under controlled conditions at 5 \pm 3°C for a period of 90 days. At predefined intervals during the storage period, samples were collected for analysis. These intervals were strategically chosen to capture potential changes in the NLC's attributes over time. The samples were subjected to a battery of tests to assess various critical parameters, including particle size, polydispersity index, zeta potential, and physical characteristics. To enhance precision, each analysis was conducted in triplicate, and the outcomes were averaged.

Statistical Analysis

The trials were conducted with a minimum of three independent repetitions to ensure data reliability. Subsequently, the results were expressed as the mean value \pm standard deviation (SD). The data underwent a one-way analysis of variance (ANOVA) to assess the level of significance in variations among the experimental groups. To determine specific pairwise differences between groups, the Tukey's post hoc test was employed, allowing for a comprehensive examination of group means. In this analysis, a significance level of $p < 0.05$ was employed to establish statistical significance. Variations meeting this standard were deemed statistically notable.

Results

The results presented in Table 2 demonstrate a range of particle sizes, spanning from 155.6 \pm 9.2 nm to 243.5 \pm 4.5 nm. The variance in particle size was caused by the manipulation of surfactant concentration.

Table 2. Characterization of NLC containing RO

Formulation	Particle Size	Polydispersity Index	Zeta Potential	Encapsulation Efficiency (%)
F1	155.6±9.2	0.21±0.05	-22.5±0.3	62.5±5.3
F2	186.2±8.5	0.15±0.35	-28.6±0.7	75.3±6.1
F3	243.5±4.5	0.17±0.07	-26.5±1.2	76.7±2.1

In our study, all NLC formulations demonstrated a negative charge, ranging from -22.5 to -28.6 mV (refer to Table 2), indicating favorable colloidal stability. This observation is in line with prior research suggesting that the selective absorption of hydroxyl ions at the oil-water interface contributes to the negative zeta potential of these formulations.

Our findings, as depicted in Table 2, show that across all NLC formulations, the polydispersity index values ranged from 0.15 to 0.21. These outcomes affirm that the NLC systems possess a desirable size distribution profile, characterized by notably narrow dispersity.

The encapsulation efficiency of quercetin within the NLC formulations varied between 62.5% and 76.7% (Table 2). This range in encapsulation efficiencies can be attributed to the concentrations of surfactants used in the formulations. It was evident that an increase in surfactant concentration led to an enhancement in encapsulation efficiency.

Figures 1 show the amounts of quercetin absorbed by the skin after 6 hours, specifically in the SC and viable epidermis/dermis layers. The ex-vivo skin permeation studies revealed a significant increase in quercetin permeation for all prepared NLC formulations compared to RO ($p < 0.01$). Among the NLC formulations, F2 showed the highest quercetin delivery to both the SC and viable epidermis/dermis layers, with retention rates 5.45 and 8.79 times greater than RO, respectively.

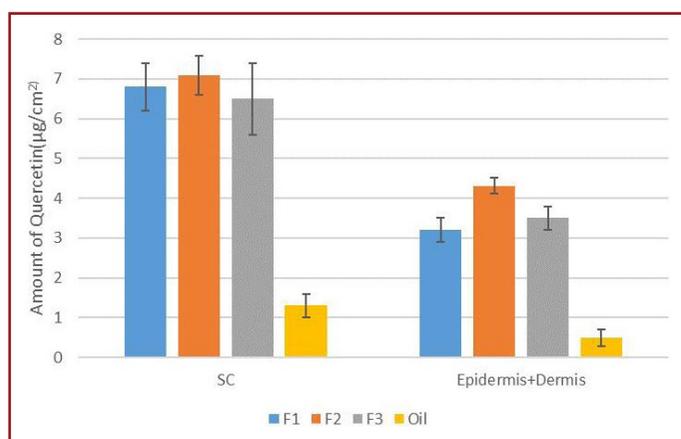


Figure 1. Quercetin amount permeated in SC and viable epidermis/dermis layers.

The stability of the selected formulation was rigorously evaluated by storing the samples at $5 \pm 3^\circ\text{C}$ for 90 days. This assessment included continuous monitoring of critical parameters to determine the NLC's stability over the storage period. Initial and periodic analyses of key attributes such as particle size, zeta potential, polydispersity index, and various physical characteristics were performed. The results of this stability assessment are depicted in Figure 2. No

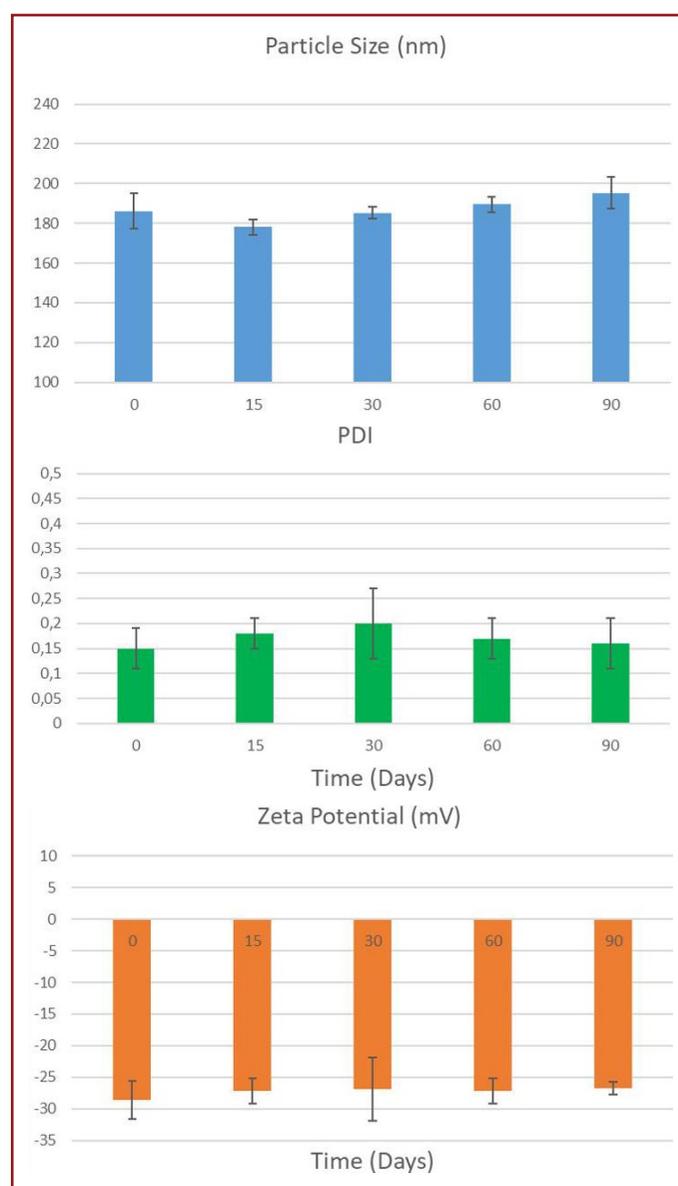


Figure 2. The result of stability studies of selected NLC formulation.

significant or statistically relevant differences were observed in the outcomes ($p > 0.05$), indicating that the selected NLC maintained its stability throughout the 90-day storage period at the specified temperature of $5 \pm 3^\circ\text{C}$.

Discussion

The characterization of NLC is crucial in understanding their potential applications in drug delivery systems. It was observed that an increase in surfactant quantity led to a proportional increase in the size of the NLCs. This phenomenon aligns with previous studies that reported similar trends^[18,19]. The correlation between surfactant concentration and particle size can be attributed to the role surfactants play in stabilizing the NLC formulation. Specifically, higher surfactant concentrations contribute to the formation of a denser interfacial layer around the binary lipid mixture. This densification effectively reduces interfacial tension between the lipid particle and the aqueous layer, resulting in smaller particles and mitigating particle aggregation^[28].

The zeta potential of nanoparticles is crucial for their colloidal stability within a dispersion. Nanoparticles with high zeta potential values, whether positive or negative, tend to resist aggregation due to the repulsive forces generated by their surface charges. All NLC formulations exhibited a negative charge, suggesting favorable colloidal stability. These findings corroborate those reported by Kim et al.^[29], who also observed a negative surface charge for NLC formulations.

Assessing the polydispersity index through dynamic light scattering is integral in determining the distribution of particle sizes within the system. A low polydispersity index value is essential as it indicates a uniform size distribution, which helps prevent aggregation within the NLC system, thereby maintaining its long-term stability. Our results, presented in Table 2, indicate that for all NLC formulations, the polydispersity index values fell within the range of 0.15 to 0.21. These findings confirm that the NLC systems exhibit a favorable size distribution profile, characterized by a remarkably narrow dispersity.

Increasing the concentration of surfactant was observed to improve encapsulation efficiency. This can be rationalized by the fact that higher concentrations of surfactants provided more effective coverage of the lipid particles, contributing to the stabilization of particles and preventing their coalescence. This, in turn, ensured the retention of the encapsulated material within the nano lipid carrier, effectively reducing the likelihood of the lipophilic active component escaping from its nano-dispersion system. These results align with prior research in the field, further supporting the correlation between surfactant

concentration and encapsulation efficiency.

To achieve a localized effect, understanding the dermal permeation process facilitated by the release of the active ingredient from the NLC is crucial. This involves penetration through the SC and targeted delivery to the viable epidermal and dermal layers. Dermal permeability evaluation was conducted using Franz-type diffusion cells, a standard method for dermal permeation tests, with pig ear skin used as a barrier. This membrane choice is popular due to its permeability and composition characteristics, which are similar to human skin. The permeation effectiveness is influenced by the physicochemical properties of the drug and the delivery vehicles^[30].

The aim was to increase quercetin concentration in the skin's outer layers while preventing systemic absorption. The permeation experiments indicated that quercetin primarily accumulated in the living epidermis and dermis, without reaching the receptor fluid, suggesting minimal systemic side effects when applied topically. Within the NLC formulations, F2 exhibited the most effective delivery of quercetin to both the stratum corneum and the viable epidermis/dermis layers. These results highlight the NLC system's effectiveness in enhancing quercetin permeation and retention within the skin layers, demonstrating F2's potential as an effective delivery method for treating skin conditions like photoaging and hyperpigmentation.

Conclusion

The NLC developed in this study, which includes RO for the first time, exhibited exceptional characteristics such as high encapsulation efficiency and reduced particle size. The formulation, prepared by combining high shear homogenization and sonication, demonstrated optimal values for particle size, polydispersity index, and zeta potential, rendering it suitable for topical application of RO. Notably, the stability of these NLCs was preserved over a 90-day period at 4°C , underscoring their potential for practical usage. The *ex-vivo* permeation studies indicated that the NLC system significantly enhanced the permeation and retention of quercetin within the skin layers, suggesting promising potential for dermatological applications. The outcomes of this study provide a foundation for further research and development of NLC-based formulations for the precise delivery of active compounds in dermatological contexts. Further investigation is essential to fully determine the capabilities of this formulation. Nonetheless, given the positive results observed to date, NLC containing RO could be considered a beneficial addition to anti-aging skincare regimens.

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