RESEARCH PAPER

# Formulation And Evaluation Of Nanostructured Lipid Carrier Loaded Topical Formulation Of Naringenin For Healing Of Wounds

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**Abstract**

Naringenin (NRG) is a plant-based flavanone that can kill microbes, work as an antioxidant, reduce inflammation, and help wounds heal. However, it has poor water solubility and limited uptake (5–8%), which limits its possible uses in medicine when applied to skin. Nanostructured lipid carriers (NLCs) are a potential way to get around these problems by making lipophilic drugs more soluble and stable and controlling their release. NLCs were made using stearic acid (a solid lipid), oleic acid (a liquid lipid), and Tween 80 (a detergent) in a study using the emulsion-sonication method. They were then prepared into a cream base for topical application. The NLC-loaded cream had good physicochemical qualities, were uniform, had a pH of 6.02 ± 0.033, viscosity of 517 ± 17.3 cp, spreadability of 46.7 ± 1.14 mm, and drug content of 96.2 ± 1.03%. In rats were used for *in vivo* excision wound studies, the wounds healed much faster—96% closure by day 10 and full contraction by day 15. These results show that NLC-based topical formulations of NRG make things more stable, release them in a controlled way, and heal wounds better. This shows that they could be used as advanced therapeutic systems for dermatological uses.

**Keywords:** Wound healing, Naringenin, Nanostructured lipid carrier, topical application, excision model

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## Introduction

When the bioactive composition of a drug is poorly stabilized, low solubility, dose-limiting side effects, a narrow therapeutic window or short half-life that makes it difficult to maintain the proper concentration of the drug over a period of time, the choice of the right drug delivery system is an important way to address these issues[1]. Previously various carrier systems including cell-penetrating peptides, hydrogels, microsponges, polymeric films, ionic liquids etc. have been reviewed for improving the delivery of such drugs[2].

Topical drug delivery is a very important part of modern medicine. It lets you treat a specific area of the body where the medicine is applied. This method works especially well for problems with the skin and other diseases that change certain parts of the skin or mucous membranes[3]. By giving medicines directly, the risk of systemic side effects is lowered. This is very important when treating conditions that are only present in one place so that the whole body doesn't have to take the drug unnecessarily. Also, avoiding hepatic metabolism by not taking the drug orally can help the drug get into the body and lower the chances of metabolic problems, which leads to better treatment results[4]. Topical versions, such as creams, gels, and patches, are easier to use and more convenient, which helps people stick to their treatment for chronic conditions. Patients find them easier to apply, so they use them more often, which leads to better outcomes[5]. Topical drug delivery gets more of the drug to the right place than systemic release, which helps the drug work better and more quickly. This targeted delivery reduces systemic absorption, which lowers the chance of toxicity and possible drug interactions. For example, antibiotics and antifungals that are applied to the skin can treat superficial skin diseases without affecting the whole body or causing as many side effects[6].

Nanostructured lipid carriers (NLCs) have changed the way drugs that are applied to the skin are made, fixing problems that come with older methods. NLCs make poorly water-soluble drugs more soluble, increase the amount of drug that can be loaded into a delivery system, and protect drugs from breaking down so they stay stable during storage and use. They help with controlled release, which keeps the drug within the therapeutic window[7], ensuring that the benefits last for a long time and that side effects are less likely to

happen. The nanostructure of NLCs helps drugs get through the layers of skin more easily, which makes the drugs more effective at the target spot. Also, NLCs let you make the kind of mixture you want because they are flexible and can include different lipids, surfactants, and drugs. Importantly, they can be tailored for site-specific drug delivery, targeting certain areas of skin or diseases. This is especially useful in dermatology[8].

Naringenin (NRG) is a plant flavonone that has been investigated widely for antimicrobial, antioxidant, anti-inflammatory, wound healing and other actions. The bioavailability of NRG ranges from 5-8%. The low aqueous solubility of NRG presents added hindrance in the topical delivery for wound healing and other conditions. The role of NLCs in improving stability of drugs, and bioavailability has been proven from literature[9]. In continuation to our previous work[10], we herein, formulation of cream loaded with NLCs containing NRG and the effect of formulation on treatment of excision wound.

**Material and Methods**

Naringenin (NRG) was purchased from Yucca Enterprises, Mumbai; Oleic acid, PEG600, Tween 20, Tween 80 and Paraffin oil were purchased from CDH.

**Preparation of NLC loaded with NRG**

Stearic acid was used as the solid lipid for preparation of NLCs. Oleic acid was used as the liquid lipid and Tween 80 as the surfactant. For preparation of NLC, weighed quantity of stearic acid (100 mg) was melted at 80°C in a clean beaker. Separately, Oleic acid (5 mg) was dissolved in ethanol and Tween 80 (10 mg) was dissolved in deionized water (5 ml) (Table 1). To the molten stearic acid was added oleic acid and NRG (5 mg). Finally, solution of Tween 80 maintained at 80°C was added drop-wise to the lipid phase and stirred for 10 min. The mixture was then sonicated at 25°C using probe sonicator at pulse of 2 sec on and 3 sec off for 5 min[10].

**Preparation of NLC loaded cream**

Cream loaded with NLC of NRG were formulated using stearic acid as the emulsifier and thickener and liquid paraffin as the oil phase using fusion method. The composition of the cream is presented below (Table 1). Accurately weighed quantities of stearic acid, span 20 and liquid paraffin were mixed and heated to 70-75°C in a clean beaker. To this was added the accurately weighed quantity of NLC loaded with NRG[11]. In a separate beaker the aqueous phase was prepared by mixing the required quantity of water and glycerine. The aqueous phase was heated to 70-75°C and mixed slowly with stirring to the oil phase. The stirring was continued further for 10 min. The prepared cream was transferred to a clean container and stored in refrigerator till use.

**Table 1. Batch formula for NLC loaded gel**

| Ingredient | Quantity per 5g | Role |
|---|---|---|
| NLC (g) | 0.5 mL | Active Ingredient |
| Stearic acid | 0.25 g | Emulsifier and Thickener |
| Span 20 | 0.1 g | Emollient |
| Liquid Paraffin | 0.25 mL | Oil Phase |
| Glycerine | 0.5 mL | Humectant |
| Water (mL) | 5 mL | Solvent |

**Evaluation of cream**

***Homogeneity and grittiness***

The cream formulation was evaluated for homogeneity by visual inspection after the cream were well set in the container. They were observed for their appearance and presence of any aggregates. The formulation was further visualized under a light microscope for the presence of particulate matter. The absence of particles fulfils the criterion for a good cream formulation.

***pH and viscosity***

1 gram of cream was dissolved in 100 ml of distilled water and allowed to stand for 2 h. The pH of the resulting solution of each formulation was measured using digital pH meter in triplicate and average values were calculated. The measurement of viscosity of the prepared cream was done with a Brookfield Viscometer. The cream was rotated at 20 rpm using spindle no. 64 and the corresponding dial reading was recorded as the viscosity values. The viscosity was measured in centipoises (cp).

***Spreadability***

The spreadability of the cream was determined using Arvouet-Grand Method[12]. Briefly, 1 g of the cream was pressed between two 20 X 20 cm horizontal plates. A weight of 125 g is placed on the upper plate for 1 min and diameter of spreading of cream was recorded. The spreadability of formulations was measured in triplicate and the average value was determined.

***Drug content***

100 mg of cream was dissolved in 100 mL phosphate buffer pH 7.4 and shaken using a mechanical shaker for 2 h to dissolve the contents completely. The solution was then filtered and the drug content was determined spectrophotometrically at 288.0 nm using a blank solution (phosphate buffer pH 7.4).

***In-vitro Drug Diffusion Study***

*In-vitro* drug diffusion study of the cream formulation was performed by using Franz-diffusion cell. The egg membrane was used in drug diffusion study and mounted in between the receptor and donor compartment of the Franz-diffusion cell. The receptor compartment contained 10 mL of phosphate buffer pH 7.4 and maintained the temperature at 37 ± 1°C. The assembly was kept in a fix position on a magnetic stirrer. 0.1 g quantity of cream sample was placed over the egg membrane and solution of phosphate buffer pH 7.4 in the receptor compartment was stirred continuously using magnetic bead at 50 rpm. Samples of 1 mL were withdrawn at 1, 2, 4, 4, 10, 10 and 12 h and diluted with 10 ml of blank solution (phosphate buffer, pH 7.4) and analyzed using spectrophotometer at 288.0 nm.

**Effect of cream on excision wound in rats**

**Experiment Design**

The animals were divided in to 4 groups of 6 rat each and the experiment was designed as per Table 5.

**Table 2. Experimental design for excision model**

| Group | Nomenclature | Treatment |
|---|---|---|
| Group I | Control | Untreated |
| Group II | Vehicle Control | Blank Gel |
| Group III | Standard | Povidone iodine ointment (5% w/w) |
| Group IV | Test | NLC Cream |

All the test samples; vehicle and standard drug samples were applied topically on the wound of each of the animals daily, under sterile conditions.

**Induction of wound**

On the day of inducing wound, each animal was anesthetized by the open mask method using short exposure to diethyl ether. The hair (fur) on the back of each rat was removed by shaving using an electric shaver. The area of the wound to be created was marked on the back of the animals with methylene blue using a circular stainless-steel stencil. A full thickness of the excision wound of 1 cm in width created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. All the surgical procedures were carried out under sterile condition. After 24 h of wound creation, the ointments were applied gently to cover the wounded area once daily until complete healing. Wound area and wound contraction, were monitored on each day[13].

**Measurement of wound contraction**

The progression of wound healing was judged by the periodic assessment of the contraction of excision wounds. Wound contraction was monitored by tracing the outline of the wound on tracing sheet and then using graph sheet to calculate the area of the wound size. All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded.

$$\text{Percent wound contraction} = \frac{Healed\ area}{Total\ area}\ X\ 100$$

**Results and Discussion**

The NLC loaded with NRG exhibited particle size of 655.4 nm with a PDI of 0.409. The zeta potential of the formulation was -17.4 mV and the entrapment efficiency was found to be 80.33%. The formulation was able to release 39.5% drug in 12 h.

**NLC loaded cream formulation**

NLC loaded oil in water cream formulation was prepared by dispersing liquid paraffin in distilled water with the help of Span 20. Stearic acid helps in providing the creamy texture to the formulation. It is a simple base cream that acts as carrier for the NLC. The NLC7 was incorporated in the cream base and the formulation was characterized for various properties.

The cream formulation was evaluated for appearance, homogeneity, pH, viscosity, spreadability and drug contet (Table 2).

**Table 2. Properties of NLC loaded cream formulation**

| Test | Observation |
|---|---|
| Appearance | Off-white |
| Homogeneity | Homogenous and non-gritty |
| pH | 6.02 ± 0.033 |
| Viscosity | 517 ± 17.3 |
| Spreadability | 46.7 ± 1.14 |
| Drug Content | 96.2 ± 1.03 |

The pH value of 6.02 ± 0.033 within the pH of skin (4.5-7.4) suggesting compatibility of the formulation for topical application. The results also indicate that the low viscosity of the gels can be helpful for the application of delivery to skin.

The homogeneity of all the cream was evaluated by the visual inspection after the cream was placed in the containers. The cream appeared to be homogeneous and uniform when seen by the naked eyes. Thereafter, the cream was visualized under the light microscope. No particle was appeared under the light microscope. This evaluation conformed that the cream was uniformly prepared. Additionally, no gritty particles were present in the cream.

The spreadability was determined using the Arvouet-Grand Method wherein the spread diameter under the experimental conditions is used as a measure of the stiffness or fluidity of the cream. The cream is considered to be semi stiff if the spread diameter is less than or equal to 50 mm and fluid if the diameter is more than 50 mm but less than 70 mm[14]. From the results it was found that the cream formulation was semi stiff in nature and suitable for topical application.

The *in vitro* diffusion of NRG from the NLC loaded cream was studied using Franz diffusion cell. Freshly peeled egg membrane was used to simulate the skin characteristics for diffusion study. The maximum amount of drug was released from the formulation was 35.2% by the end of 12$^{th}$ hour of the study (Figure 1).

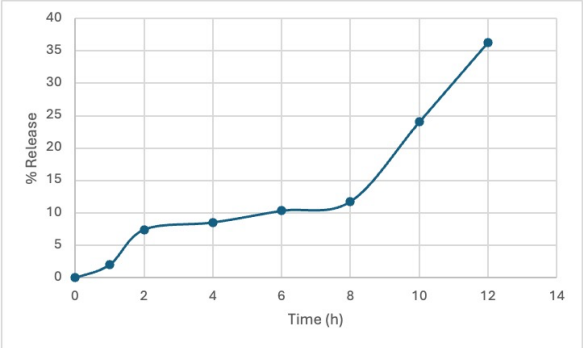


**Figure 1. Cumulative release plot of NRG from NLC loaded cream**

As seen from the results, the cream formulation was able to release almost equivalent amount of drug in 12 hours as released from the NLC, suggested proper incorporation and release of drug from the formulation.

**Wound healing action**

The NLC loaded cream formulation was evaluated for the *in vivo* wound healing effect by the excision model (n=6). The topical application of 5 % w/w of the NLC loaded cream resulted in an enhanced and statistically significant ($p < 0.001$) wound healing activity *in vivo*. The wound area measurements of the progressive healing of the excision wounds for the control; cream control; standard reference drug and NLC loaded cream are presented in table 3. From the results it can be clearly seen that the NLC loaded cream exhibited an excellent wound healing potential with almost complete closure (96%) of the wound of the animals by 10 days. The NLC loaded cream exhibited 100 % contraction of wound on the 15$^{th}$ day whereas only 82.69 % contraction of wound was found in the control animals after 21 days (Figure 2).

**Table 3. Area of wound in excision model**

| Group | Area of wound (cm$^2$) | | | | |
|---|---|---|---|---|---|
| | Day 0 | Day 5 | Day 10 | Day 15 | Day 21 |
| I | 1.030 ± 0.038 | 0.9234 ± 0.089 | 0.5794 ± 0.070 | 0.345 ± 0.089 | 0.1783 ± 0.061 |
| II | 1.0135 ± 0.024 | 0.590 ± 0.077 | 0.3266 ± 0.096 | 0.085 ± 0.044 | 0.0 ± 0.0 |
| III | 1.0134 ± 0.016 | 0.3116 ± 0.115 | 0.0183 ± 0.017 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| IV | 1.0201 ± 0.018 | 0.3116 ± 0.115 | 0.0383 ± 0.029 | 0.0 ± 0.0 | 0.0 ± 0.0 |

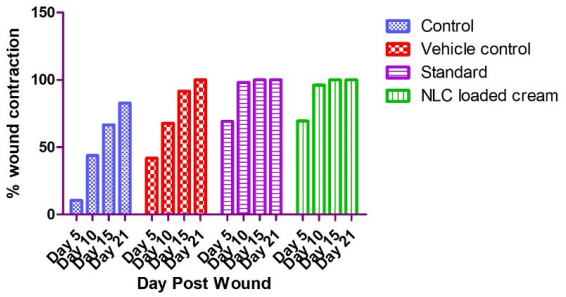


**Figure 2. % contraction of wound exhibited by various groups**

**Conclusion**

The present study successfully developed and evaluated a nanostructured lipid carrier (NLC)–based topical cream formulation of naringenin (NRG) for wound healing. The optimized NLCs demonstrated favorable physicochemical properties, including nanoscale particle size, acceptable polydispersity, negative zeta potential, and high entrapment efficiency, ensuring stability and sustained drug release. Incorporation of NLCs into a cream base yielded a formulation with desirable attributes such as homogeneity, skin-compatible pH, appropriate viscosity, and good spreadability, confirming its suitability for topical application.

In vitro diffusion studies revealed controlled release of NRG from the cream, while in vivo excision wound models in rats demonstrated significantly enhanced wound healing compared to control and vehicle groups. The NLC-loaded cream achieved nearly complete wound closure within 10 days and full contraction by day 15, outperforming untreated controls and showing comparable efficacy to standard treatment.

Overall, the findings establish that NLC-based topical delivery of naringenin effectively addresses its limitations of poor solubility and low bioavailability, while providing sustained release and superior therapeutic outcomes. This formulation represents a promising, biocompatible, and patient-friendly

approach for wound management and may be extended to other dermatological applications. Future studies should focus on long-term stability, scale-up, and clinical evaluation to translate these results into practical therapeutic use

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