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Green Chemistry-Driven Development of Topical Vitamin D3 Nanostructured Lipid Carriers: *In Silico* Lipid Selection and Anti-Inflammatory Evaluation

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ABSTRACT

Vitamin D3 exhibits promising anti-inflammatory activity; however, its topical application is limited by poor aqueous solubility and low dermal bioavailability. Nanostructured lipid carriers (NLCs) are biocompatible lipid-based systems that enhance cutaneous delivery. This study optimized the lipid composition of Vitamin D3-loaded NLCs using an integrated green chemistry approach that combined *in silico* screening and experimental validation. Molecular docking and physicochemical compatibility prediction identified monostearin and Miglyol 812 as optimal lipid candidates. NLCs were then prepared at different solid–liquid lipid ratios (F1: 7:3, F2: 8:2, F3: 9:1) using an environmentally benign, organic-solvent-free high-shear homogenization process and were stabilized with a low concentration (3%) of non-ionic, low-toxicity surfactants (Tween 60 and PEG 400). *In vitro* release was assessed using UV–Vis spectrophotometry, and anti-inflammatory activity was evaluated using a carrageenan-induced paw edema model in rats. *In vivo* evaluation confirmed that formulation F1 produced the greatest reduction in paw edema (3.94% at 6 h), approaching the activity of the positive control and markedly outperforming the negative control. Overall, the combined *in silico*–experimental strategy improved the performance of vitamin D3-loaded NLCs while reducing material use and supporting environmentally benign formulation development.

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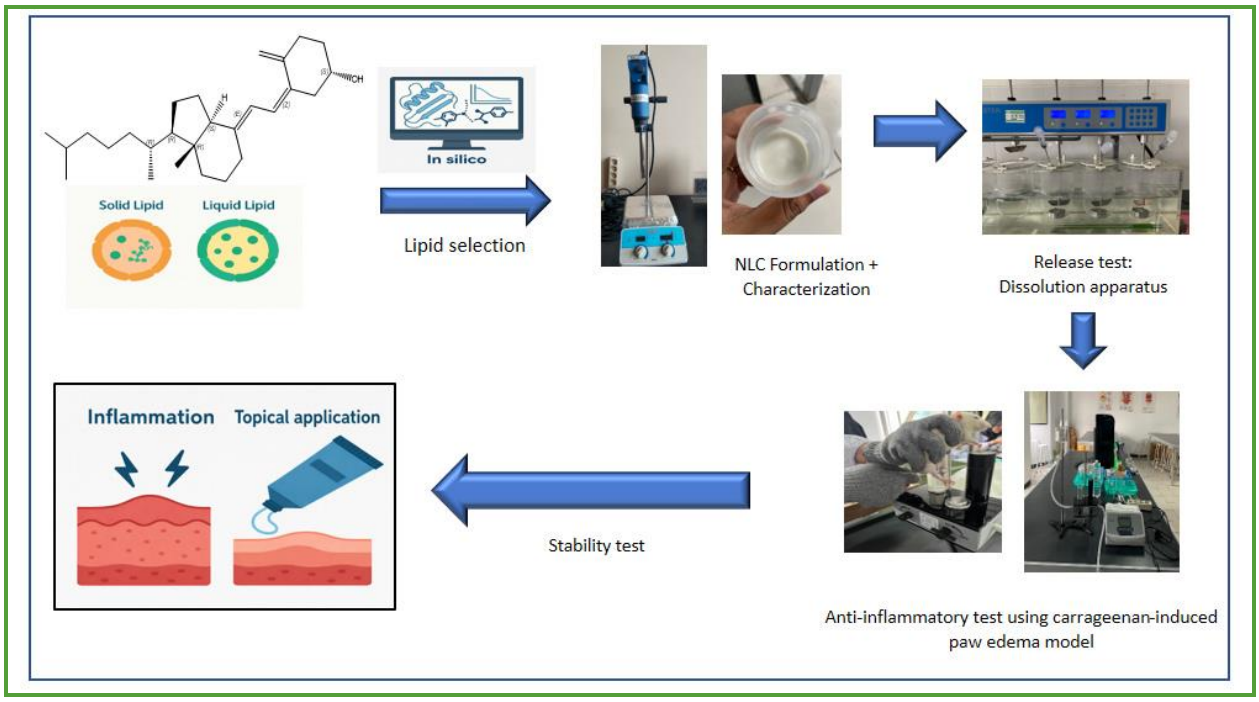
Graphical Abstract



Introduction

Changes in the skin's defense mechanisms, impaired barrier function, or exposure to external stimuli can initiate inflammatory skin diseases that alter epidermal structure and function [1,2]. These conditions are sustained by complex interactions among multiple cell types, particularly keratinocytes, which actively produce adhesion molecules and pro-inflammatory cytokines, including TNF-α, IL-1α, and IL-1β. Inflammation represents a protective response to infection or injury and is characterized by redness, heat, swelling, and pain. Activation of the innate immune system through Toll-like receptors (TLRs) triggers MyD88- and TRIF-dependent signaling cascades that activate NF-κB, leading to transcription of pro-inflammatory mediators such as iNOS, IL-6, and TNF-α [3]. Vitamin D3 (cholecalciferol) has garnered significant attention due to its immunomodulatory and anti-inflammatory properties, which extend beyond its classic role in calcium and bone homeostasis. Recent reviews indicate that active vitamin D3 and its analogs act through the vitamin D receptor (VDR) to inhibit NF-κB signaling and reduce the production of pro-inflammatory cytokines such as IL-6, IL-1β, and TNF-α in various immune cells, thereby modulating innate and adaptive immune response [4]. Despite its therapeutic potential, the clinical application of vitamin D3 is limited by its low water solubility and lipophilicity, which reduce its absorption, its unstable degradation when exposed to environmental factors, and its low dermal bioavailability when administered topically. Therefore, to maximize its potential in inflammatory disorders, an effective delivery system such as a nanostructured lipid carrier is required to enhance solubility, protect the molecule, and improve skin penetration [5]. Nanostructured lipid carriers (NLC) have gained significant attention as a second-generation delivery system capable of overcoming the formulation challenges of lipophilic drugs such

as vitamin D3. Recent studies have shown that lipid nanoparticle and NLC formulations can enhance the stability of vitamin D3 molecules, achieve high encapsulation efficiency, and improve skin penetration. For example, research on vitamin D-loaded lipid nanoparticles reported a particle size of approximately 153.9 nm, a zeta potential of –54.3 mV, and an encapsulation efficiency of approximately 96.98%, with improved skin penetration when integrated into topical creams [6]. Previous research on NLC vitamin D3 with solid and liquid lipid ratios demonstrated an encapsulation efficiency of approximately 82% and physicochemical characteristics suitable for topical application [7]. Another formulation using rhamnolipid as a surfactant successfully produced NLC with a small size (approximately 90-100 nm) and high stability, thereby improving the bioaccessibility of vitamin D3 [8]. Based on recent reviews, NLC is a promising solution for enhancing the solubility, bioavailability, and skin penetration of vitamin D3 for topical therapeutic use [9]. The choice of lipid excipients is one of the most crucial elements influencing the functionality of NLC [10]. Particle size distribution, stability, release behavior, and encapsulation efficiency are all significantly affected by the physicochemical compatibility between the drug and the lipid matrix [11,12]. For instance, it has been demonstrated that changes in the type and proportion of solid and liquid lipids can significantly alter drug release patterns, skin penetration, and encapsulation effectiveness. Lipid selection has historically depended on an empirical trial-and-error method, which is labor-intensive, time-consuming, and expensive in terms of experimental resources [12,13]. Recent research has explored computational tools, such as molecular docking and *in silico* prediction models, to overcome these limitations. These tools provide a more effective and eco-friendly method of finding lipid-drug combinations that work while lowering the environmental impact and experimental burden [14].

The capacity of computational *in silico* methods, such as compatibility prediction and molecular docking, to predict drug–lipid interactions before laboratory formulation has drawn increasing attention [13]. These methods facilitate sustainable pharmaceutical development by enabling the rapid identification of optimal excipients, reducing material consumption, and minimizing experimental waste [8]. However, there are still a few publications that combine experimental validation of NLC formulations with *in silico* lipid screening, especially for vitamin D3 [12]. Thus, the goal of this investigation was to create and enhance vitamin D3-loaded NLCs using a comprehensive approach that combined experimental evaluation and *in silico* lipid selection [7]. The solid and liquid lipids with the highest affinity for vitamin D3 were identified using molecular docking and physicochemical predictions. These lipids were then formed into NLCs using the high-shear homogenization process [13]. The compositions' anti-inflammatory properties, both *in vivo* and *in vitro*, were systematically assessed [15,16]. In line with the principles of green chemistry, this work proposes a sustainable formulation approach that enhances the efficacy of vitamin D3 topical delivery systems, accelerates lipid screening, and reduces experimental effort [17]. NLC is a system that ranges in size from 100 to 1,000 nm, based on lipids, utilizing a combination of solid and liquid lipid matrices that can be stabilized by the addition of surfactants. The use of NLC can improve skin hydration, leading to increased intercellular gaps between corneocytes and thereby facilitating drug penetration into the skin [14]. This study utilized Wistar strain rats because they are sensitive to a high-fat diet. The Wistar

strain rats used were male, as they can provide more stable research results, given that they are not influenced by the estrus cycle and pregnancy. Rats are also easier to control in terms of food intake and physical activity, thereby minimizing bias during the study [18,19]. Based on the above description, the purpose of this study is to determine whether vitamin D3 has anti-inflammatory activity in male Wistar strain white rats.

Experimental

Material and methods

Materials and equipment used in the study, as described in Kristiainingasih *et al.*, included vitamin D3, Monostearin, Myglyol 812, Tween 20, PEG 400, phosphate buffer pH 6.0, carrageenan, ketamine, and tools such as the Ultra Turrax IKA T-25, magnetic stirrer, hot plate, animal balance, plethysmometer, pH meter, particle size analyzer (PSA) (Delsa™Nano), and cone and plate viscometer. This research is a continuation of Kristianingsih's research and focuses on the use of *in silico* for lipid selection using Autodock 4.0 software, NLC vitamin D3 release testing, and anti-inflammatory activity testing. NLC was produced using the high shear homogenization method [7].

Preparation of docking

Autodock 4.2 software was used to conduct molecular docking investigations of vitamin D3 using lipids. In research, these seven types of lipids were used with different groups (monostearin, and cetyl palmitate) and liquid lipids, which have long and varied chains (Miglyol 812 and Transcutol). Solid and liquid lipids is used to perform docking studies to predict the solubility and interactions between vitamin D3 and selected lipids. In the initial stage, tests and evaluations were carried out through laboratory experiments (study of the solubility of vitamin D3 with liquid lipids). The results obtained from the docking studies were compared with those of laboratory experiments to determine the degree of validity, accuracy, and reliability of the docking method used to predict the solubility of vitamin D3 in lipids. The results of molecular docking include the RMSD (root mean square deviation) value and binding free energy (ΔG) [12].

Preparation of NLC

NLC preparation using the high shear-homogenization method, as described in Kristianingsih report [7]. This high-energy technique is widely employed for the preparation of nano-dispersions. Lipophilic actives are incorporated into a molten lipid matrix to prevent premature recrystallization, after which a pre-heated aqueous surfactant phase is added and emulsified. The mixture is subsequently subjected to high-speed homogenization to achieve nanoscale dispersion. The method is broadly adopted due to its operational simplicity and reproducibility [20].

In vitro vitamin D3 release

The *in vitro* release study was performed using a dissolution tester. The dissolution medium was maintained at 32 ± 0.5 °C to simulate skin temperature. A weighed amount of the NLC formulation was placed into a sample disk and covered with a membrane ensuring the absence of air bubbles. The disk was positioned at the bottom of the vessel with the release surface facing upward and aligned with the paddle, which was kept at a distance of 25 ± 1 mm. The medium volume was set at 500 mL and the paddle was operated at 100 rpm. Aliquots of 5 mL were withdrawn at predetermined

intervals (10–180 min) from the midpoint between the medium surface and the paddle, and each withdrawal was replaced with an equal volume of phosphate buffer pH 6.0 ± 0.05 at the same temperature. The samples were analyzed by UV–Vis spectrophotometry at the predetermined analytical wavelength. Dilution was corrected using the Wurster equation. The results of the calculations formed a curve showing the relationship between the cumulative amounts of vitamin D3 released (μg/cm^2) and the root of time. From the resulting curve, a regression equation could be created. Based on Higuchi's law, the slope of the regression equation represents the rate of release (flux) of vitamin D3 from the base [21].

Anti-inflammatory

Preparation of animal study

The test animals used in this study were 15 male white rats weighing 100-250 g. All rats were acclimatized for seven days to adapt to the environment before being treated. Test animals were considered healthy if, during observation, they did not show body weight deviation (>10%) and visually exhibited normal behavior.

Preparation of the solution

Carrageenan 1% was prepared by weighing 100 mg of carrageenan, then it was placed in a 10.0 mL measuring flask and filled with 0.9% NaCl solution to the mark, and incubated at 37 °C for 24 h.

Anti-inflammatory study

Rats are divided into three treatment groups. Each mouse is weighed, marked on the left foot, and the volume of the left foot is measured using a plethysmometer and recorded as Initial Volume (Vo). After that, the paw was injected with 0.1 mL of 1% carrageenan. 1 h after carrageenan induction, the rats were anesthetized using ketamine at a dose of 20 mg/kg BW, after which the preparation was administered based on the following: Group I: Administered NLC Sodium Diclofenac 1% (+). Group II: Administered NLC preparation without vitamin D3 1% (-). Group III: Administered NLC vitamin D3 preparation with the best formulation (F1). After treatment for 60 min, the left paw volume was measured again, with measurements taken every 60 min for 360 min, and changes in swelling levels were recorded as Mouse Paw Volume (Vt).

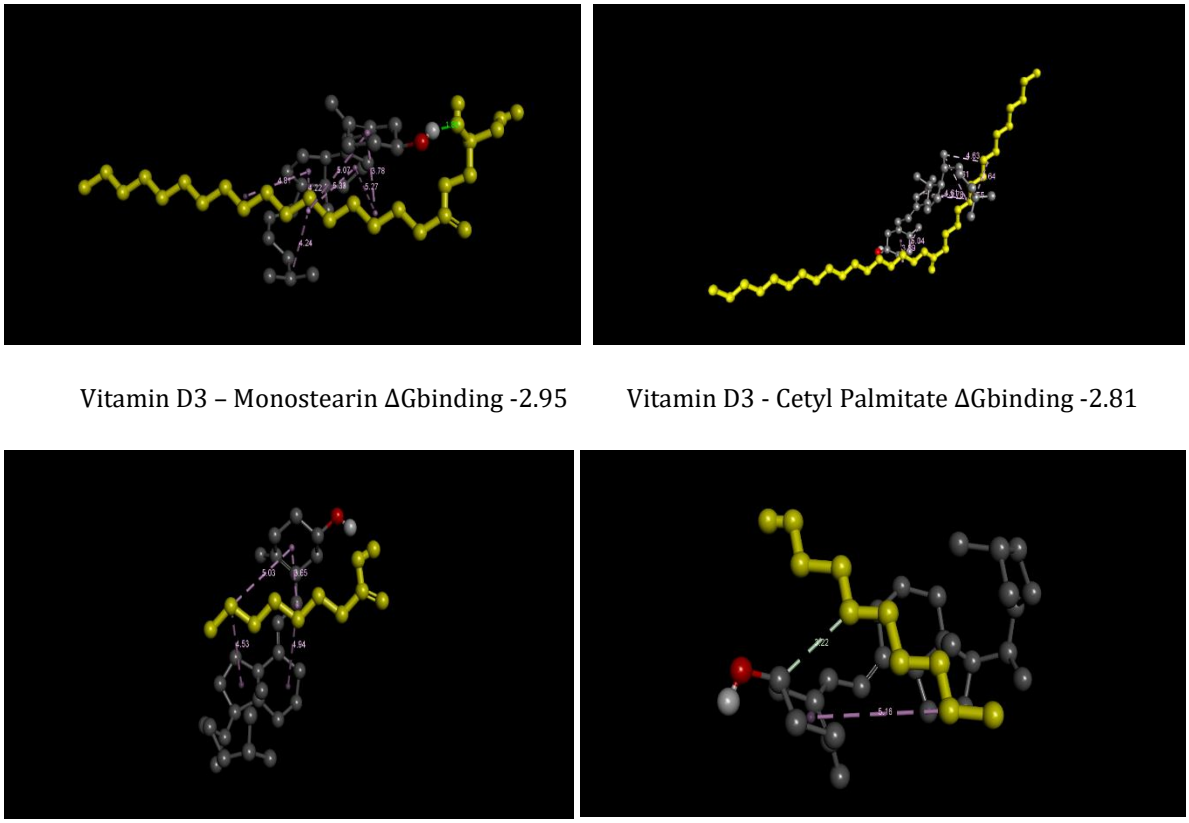
Stability test

Stability is the ability of a product to maintain its properties and characteristics, ensuring they remain unchanged from the time of manufacture (identity, strength, purity, and quality) within specified limits throughout its storage and use period (shelf life). Stability testing is carried out using the Freeze-Thaw test method. NLC samples were stored at a temperature of 30 ± 2 °C for 12 days. In the freeze-thaw method, NLC samples were stored at 4 ± 2 °C for 24 h (counted as one cycle), then repeated for up to six cycles (12 days). Organoleptic tests, pH tests, particle size, and viscosity were evaluated on the last day of storage.

Results and Discussion

Nanostructured Lipid Carrier is a lipid-based system that uses a combination of solid and liquid lipid matrices stabilized by the addition of surfactants and cosurfactants [22]. In this study, an NLC system with vitamin D3 as the active ingredient was used. Vitamin D3 is insoluble in water and exhibits poor oral bioavailability [23]. Due to a vitamin D3 deficiency, a topical preparation was formulated using the NLC system. In NLC, the presence of liquid lipids

alters the crystallization of solid lipids, allowing the active ingredient to enter and influence the characterization of the vitamin D3 NLC formulation. NLC was selected as the drug delivery system to address issues of poor water solubility and instability**.** The NLC system was created using monostearin as solid lipids and Miglyol 812 as liquid lipids with varying ratios (F1 7:3, F2 8:2, and F3 9:1). The solid lipids contained in the system will carry more bioactive components. In addition, solid lipids can also trap bioactive compounds more effectively. The ratio of solid lipids, liquid lipids, and surfactants significantly affects the stability of the resulting NLC system. The addition of high levels of solid lipids can affect trapping efficiency, whereas the addition of water to the NLC system can help stabilize it during the manufacturing process [24]. *In silico* testing in this study aims to identify the optimal lipid forms by examining the interactions between lipids and vitamin D3. There are two selected lipids (Monostearin, cetyl palmitate) and two liquid lipids (Myglyol 812 and Transcutol). The best results are based on the ΔG value (free binding energy), as a low ΔG indicates a low energy of bonding, resulting in high solubility. The ΔG values are listed in Table 1, and the *in silico* results are shown in Figure 1. Based on these results, the selection of solid and liquid lipids is appropriate.



Vitamin D3 – Monostearin ΔGbinding -2.95

Vitamin D3 - Cetyl Palmitate ΔGbinding -2.81

Vitamin D3 – Miglyol ΔGbinding -1.06

Vitamin D3 – Transcutol ΔGbinding -0.86

Figure 1. Molecular interaction profile of vitamin D3 and NLC lipid candidates

Table 1. Result docking with solid and liquid lipid

Lipid group	Chemical name	ΔGbind (kcal/mol)
Triglycerides	Monostearin ($C_{21}H_{42}O_4$)	-2.95
Wax	Cetyl phalmitate	-2.81
Medium-chain triglyceride (MCT)	Miglyol 812 ($C_{29}H_{54}O_6$)	-1.06
diethylene glycol monoethyl ether	Transcutol	-0.86

In silico testing in this study aims to identify the optimal lipid forms by examining the interactions between lipids and vitamin D3. Two selected lipids are monostearin and Miglyol 812 [7]. These preparations are then subjected to a release test. The NLC vitamin D3 release test aims to determine how much vitamin D3 can be released from the base. This release test uses the dissolution method. Drug release analysis was conducted using a UV–Vis spectrophotometer to quantify the amount of vitamin D3 released into the dissolution medium. The procedure began with determining the maximum absorption wavelength and developing a standard calibration curve in the release medium. Vitamin D3 exhibited a maximum absorbance at 264 nm. The calibration curve constructed from standard solutions (10–50 ppm) yielded a regression equation of **y =** 0.1078x + 0.0162, with an R^2 of 0.9966, demonstrating excellent linearity and confirming the method's validity for quantitative analysis. The *in vitro* release study of vitamin D3 was subsequently carried out using a USP Dissolution Apparatus V. The dissolution medium consisted of 0.5% Tween 80 and 20% ethanol in phosphate buffer pH 6.0, selected to ensure adequate solubility of vitamin D3 while maintaining a pH comparable to skin conditions. The presence of a small amount of surfactant in the medium facilitated the release of this poorly water-soluble compound. Release samples (5 mL) were withdrawn at predetermined time intervals and analyzed at 264 nm. Interference studies confirmed that the components of the release medium did not affect absorbance at this wavelength, enabling accurate quantification of vitamin D3 throughout the release study. The release test was conducted to evaluate the release profile and release rate (flux) of vitamin D3 from the NLC system. The release profile is illustrated as the cumulative percentage of vitamin D3 released over time (Figure 2), while the release rate (flux) is represented by the slope of the regression line obtained from the cumulative release versus time plot.

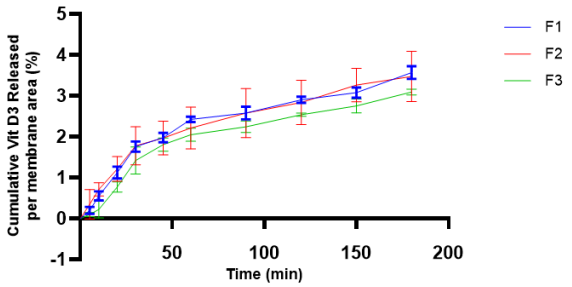


Figure 2. Determination of cumulative vitamin D3 release ((μg/cm²/time½)

To determine the release kinetics, the vitamin D3 release data were fitted to several mathematical models. The linearity of the calibration curve (r > r-table, 95% confidence level, α = 0.05) confirmed the validity of the UV–Vis method for quantifying vitamin D3. The mathematical models evaluated included the zero-order, first-order, and Higuchi diffusion models. The regression equations and correlation coefficients (r) for each model are summarized in Table 2. Among the models tested, the Higuchi model exhibited the highest correlation coefficient, indicating that vitamin D3 release from the NLC matrix follows Higuchi diffusion kinetics.

Table 2. Kinetic model of drug release

Kinetic model of release		F1	F2	F3
Zero order	Regression equation	Y= 0.0178 + 0.5034	y = 0.0177x + 0.5464	y = 0.0165x + 0.2849
	r	0.8644	=0.8928	0.8596
First order	Regression equation	y = 0.0043x – 0.2842	y = 0.0037x – 0.2156	Y= 0.0052x – 0.4621
	r	0.5082	0.5923	0.4493
Higuchi	Regression equation	y = 0.3634x – 0.3484	Y=0.3506x – 0.2507	y = 0.3327x – 0.4517
	r	0.9816	0.9895	0.9748

Based on the 3-h release data, the flux values for F1, F2, and F3 were 0.3581 ± 0.0042, 0.3306 ± 0.0083, and 0.3193 ± 0.0189, respectively (Table 3). A one-way ANOVA revealed no statistically significant difference among the flux values of the three formulations ($p = 0.757$; $p > 0.05$), indicating that the release rates of all formulations were comparable.

Theoretically, formulations containing higher proportions of liquid lipids (such as F1) are expected to exhibit faster release due to increased molecular mobility within the lipid matrix (Apostolou *et al.*). In this study, F1 demonstrated the highest release rate (approximately 0.35 or 35%), consistent with its higher liquid-lipid content compared with F2 and F3. Previous research reported that vitamin D3 incorporated into an NLC system showed approximately 9.3% release [25]. Although a trend toward higher liquid-lipid ratios was observed, differences among F1, F2, and F3 were not statistically significant. It is possible that more pronounced differences in release behaviour would emerge if the lipid ratios were varied more broadly. The study proceeded to evaluate the anti-inflammatory activity of the vitamin D3-loaded NLC formulations in male Wistar rats. Five treatment groups were used (Table 4), consisting of a positive control (NLC Sodium Diclofenac), a negative control (NLC without vitamin D3), and three test formulations (F1, F2, and F3). NLC without vitamin D3 was selected as the negative control because it does not exhibit anti-inflammatory activity, whereas NLC Sodium Diclofenac served as the positive control due to its established and potent anti-inflammatory effect.

Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID) that primarily inhibits cyclooxygenase (COX) activity, thereby preventing the conversion of arachidonic acid to pro-inflammatory prostaglandins. In carrageenan-induced inflammation, diclofenac sodium is particularly effective in suppressing the second phase of the inflammatory response. Male Wistar rats were chosen as the experimental animals because their physiological conditions are more stable than those of females, whose responses can be influenced by the estrous cycle. To minimize biological variability, the test animals were standardized to a body weight of 150–250 g and an age range of 2–3 months. The animals were randomly divided into five groups, with three rats in each group (Figure 3). The number of animals per group was determined based on the Resource Equation method, ensuring adequate statistical power while adhering to ethical considerations. In addition to AUC, the percentage of inflammatory inhibition was calculated to show the extent to which a formula reduces the inflammatory response relative to the negative control. This value provides easier information than simply looking at the volume/percentage of oedema at each observation time. Based on the calculation results, the negative control showed the highest AUC value (244.33 %·h), indicating the highest

level of inflammation. The positive control (NLC sodium diclofenac) had an AUC (188.90 %·h) with an inhibition percentage of 22.68%, consistent with the anti-inflammatory profile of the drug. Formula F1 showed the smallest AUC value (125 %.h) and the highest percentage of inhibition (48.85%), indicating that F1 is the most effective formula in suppressing carrageenan-induced edema. Formula F2 also provided significant inhibition (45.45%) with an AUC of 133.34 %·h, while F3 showed inhibition of 32.93% with an AUC of 163.89 %·h. This indicates that increasing the proportion of liquid lipids in the NLC system could enhance the release and anti-inflammatory activity of Vitamin D3. The AUC and % inhibition results can be seen in the Table 5.

Table 3. Vitamin D3 release rate from the NLC system according to the Higuci model

Formula	Replication	Release rate	Average ±SD
F1	1	0.3618	0.3581±0.0042
	2	0.3535	
	3	0.3591	
F2	1	0.3212	0.3306±0.0083
	2	0.3367	
	3	0.334	
F3	1	0.3198	0.3193±0.01890
	2	0.3002	
	3	0.338	

Table 4. Evaluate the anti-inflammatory activity of the vitamin D3-loaded NLC with paw edema

Treatment group	V0	Vt	t1	t2	t3	t4	t5	t6	Reduction (Vt–t6)
Positive control (NLC Diclofenac 1%)	1.8	2.9	2.8	2.6	2.5	2.3	2.1	2	0.9
Negative control (Blank NLC 1%)	1.7	2.7	2.6	2.5	2.5	2.4	2.3	2.2	0.5
F1 (NLC vitamin D3 1%)	1.8	2.7	2.6	2.3	2.2	2.1	2	1.9	0.8
F2 (NLC vitamin D3 1%)	1.8	2.6	2.5	2.4	2.3	2.1	2	2	0.7
F3 (NLC vitamin D3 1%)	1.8	2.6	2.6	2.5	2.5	2.3	2	1.9	0.7

Note: *V_0 = paw volume before carrageenan induction; V_t = paw volume at 1 h after induction; t_1–t_6 = paw volume measured at 1–6 h post-treatment; and Reduction (V_t–t_6) = decrease in paw volume from peak edema to hour 6.*

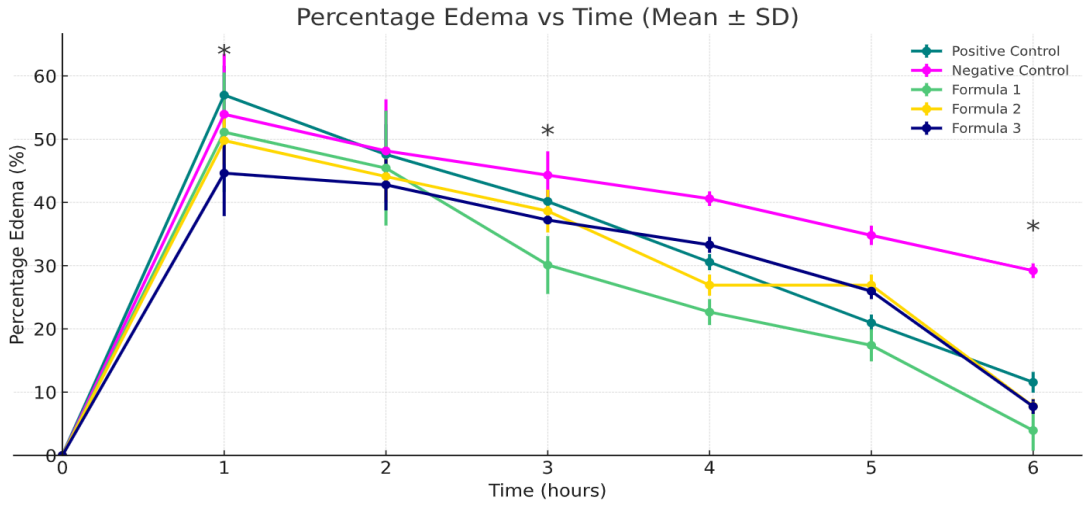


Figure 3. The area under the curve (AUC) calculation is performed to describe the overall inflammatory response during the observation period. The AUC value reflects the area of edema that forms over time, so the smaller the AUC value, the lower the level of inflammation in the test animals. Therefore, AUC is used as a quantitative parameter to comprehensively assess anti-inflammatory efficacy

Table 5. AUC Values and anti-inflammatory percentage inhibition

Treatment	AUC (%·h)	% Inhibition
Negative control	244.33	0
Positive control	188.90	22.68
F1	125.00	48.85
F2	133.34	45.45
F3	163.89	32.93

All animal procedures were approved by the Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, Universitas Airlangga (FKH UNAIR), under ethical clearance number No: 2.KEH.50.04.2025. The *in vivo* inflammatory study was conducted in accordance with institutional guidelines for the care and use of laboratory animals and adhered to the 3R principles (Replacement, reduction, and refinement). Organoleptic evaluation over 12 days of storage showed that all vitamin D3 NLC formulations (F1, F2, and F3) remained unchanged, retaining their semisolid appearance, yellowish-white colour, and characteristic fatty odour. This indicates that the formulations were physically stable during the storage period. Particle size stability was also maintained, with all formulations exhibiting size variations of <6% despite slight fluctuations among replicates. Although the particle size distribution showed minor increases, the mean particle size of all formulations remained below 600 nm throughout storage, consistent with the desired range for effective skin penetration (Danaei *et al.*). The pH values of all formulations remained stable during the 12-day storage period, with variations <6%, suggesting no significant change in acidity. Viscosity also demonstrated <6% variation, indicating good rheological stability.

Table 6. Stability results at 12 days of storage

Parameter	After 12 days	Δ (%)
Particle size (nm)	274.3 ± 4.0	266.1 ± 3.2
PDI	0.364 ± 0.01	−4.2
Zeta (mV)	Zeta (mV)	+10.6
pH	6.0 ± 0.07	+11.1
Viscosity (cP)	405.2 ± 7.1	+10.8
Organoleptic	Homogeneous	−2.9

Minor changes in pH and viscosity may be attributable to sampling factors, instrument sensitivity, or limited particle coalescence during storage. Overall, the stability evaluation suggests that the vitamin D3 NLC formulations exhibit favourable physical stability; however, formulation refinement may be required to prolong shelf life, such as incorporating thickening agents (*e.g.,* PEG derivatives) or embedding the NLCs into semisolid or patch-based systems. The use of PEG-400 as a co-surfactant likely contributed to steric stabilization by preventing nanoparticle aggregation. Further optimization of solid–liquid lipid ratios may also enhance long-term stability, as indicated in Table 6.

Conclusion

The developed cholecalciferol-loaded NLC demonstrated suitable physicochemical properties, validated analytical performance, and good stability during storage. The system enhanced dermal penetration, with Formula 1 showing the best penetration profile. *In vivo* testing confirmed that the NLC–vitamin D_3 formulation exhibited notable anti-inflammatory activity, approaching the effect of the positive control. The results highlight the effectiveness of using a green chemistry-based formulation strategy combined with *in silico* lipid selection, which not only reduced the need for organic solvents, but also optimized lipid compatibility and drug loading. The NLCs showed controlled release behavior, suggesting potential for sustained therapeutic effects upon topical application. Compared with conventional topical formulations, these NLCs offer improved bioavailability, reduced irritation potential, and enhanced skin retention. Overall, this study demonstrates that vitamin D_3-loaded NLCs can serve as a promising platform for topical anti-inflammatory therapy, providing a balance between efficacy, safety, and environmental sustainability. Future studies could explore long-term *in vivo* efficacy, mechanistic pathways of anti-inflammatory action, and clinical translation potential.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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