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RESEARCH ARTICLE

Development and physicochemical characterisation of nanostructured lipid carriers for entrapment of vitamin D3 prepared at different lipid ratios

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Abstract

Background: Vitamin D3 plays a role in immunity, especially in the body's regulation of the inflammatory response. The limitations of vitamin D3 are poor bioavailability and water-insoluble. Nanostructured Lipid Carriers (NLC) can increase the solubility of water-insoluble active ingredients. **Objective**: To develop NLC as a delivery agent of vitamin D3 for topical application. **Method**: NLC was created using a high-shear homogenisation method with various lipid ratios. NLC uses solid lipids (Monostearin) and liquid lipids (Miglyol 812) with variations in the ratios of F1 (7:3), F2 (8:2) and F3 (9:1). NLC is expected to be able to protect the active ingredients in the lipid matrix so that the stability of the active ingredients increases. Characterisation was performed in organoleptic, pH, particle size, viscosity, zeta potential, and entrapment efficiency. **Result**: Vitamin D3 NLC system had good characteristics: pH suitable for topical use, particle size < 600nm, PI < 0.5, Zeta potential -26.80 to -33.76. F1 resulted in the highest entrapment efficiency of 82.18%. **Conclusion**: Vitamin D3 NLC systems with different lipid concentration ratios affect the physical and chemical characteristics. Increasing the liquid lipid content in the Vit D3 NLC system can reduce particle size and viscosity and increase entrapment efficiency.

Introduction

Vitamin D3 (Cholecalciferol) is a supplement to prevent and treat vitamin D deficiency (Vieira & Souza, 2022; Park, 2019). In addition, vitamin D3 is needed to help absorb calcium and phosphorus in the body. Vitamin D3 is a fat-soluble vitamin and is helpful for bone marrow development (Demirbilek *et al*., 2017). Vitamin D3 is a hormone responsible for ruling calcium homeostasis (Leal *et al*., 2020). This vitamin is particularly applicable to dermatology because the skin is a significant biosynthesis point and a target organ (Wat & Dytoc, 2014).

Vitamin D3 is a form of vitamin D (Calciferol). Vitamin D has two primary chemical forms: vitamin D2 (ergocalciferol) and vitamin D3 (Vieira & Souza, 2022). In plants, vitamin D2 is produced by UV radiation from ergosterol, whereas vitamin D3 is synthesised in the human epidermis or found in oily fish and egg yolks (Park, 2019). Although both forms show similar effects, vitamin D3 is biologically more active and more potent in increasing the storage of vitamin D content than D2 (Seo *et al*., 2019). Vitamin D3 is synthesised in the skin by photoconversion of 7-dehydrocholesterol to pro-vitamin D with the help of ultraviolet (UV) light at 280-320 nm (UV-B).

Challenges in the delivery system of vitamin D3 include being unstable in minerals, oxygen, moisture, and acidic environments and having low bioavailability (Seo *et al*., 2019). Vitamin D3 is a fat-soluble lipophilic vitamin with a log P value of 7.5 and a molecular weight of 384.6 Daltons (Vieira & Souza, 2022). The characteristics of vitamin D3 include having a melting

point of 83-86°C, being stable at pH 6, being in the form of crystals or liquids that are yellow to orange in colour, being insoluble in water and soluble in ethanol, methanol, chloroform, vegetable oil and has bitter taste (Farooq *et al*., 2019).

When Vitamin D3 is used orally, patient compliance rates are less than 60% (Bubshait *et al.*, 2018), which has resulted in several researchers looking for alternative delivery systems, one of which is topical. The effectiveness of vitamin D3 topically is of significant effect when used with potent topical steroids *(Barrea et al.*, 2017). A particular study found that Vitamin D3 could reduce oedema after seven days of administration (Almeida Moreira Leal *et al*., 2020). Prediction of the anti-inflammatory activity of vitamin D3 using the Prediction of Activity Spectra for Substances (PASS) program, which can be accessed online, has an active probability value of 0.702 (Probability to be active or Pa > 0.7), almost the same as diclofenac sodium, which means it has potential to be a candidate as an anti-inflammatory. Vitamin D3 can be predicted for cases of inflammation. Based on the data, chronic inflammatory cases can be a cause of death, and inflammatory conditions frequently contribute to more than 50% of deaths globally (Sianipar & Jap, 2023).

Nanostructured Lipid Carriers (NLC) particle sizes range from 10-1000 nm (Pardeike *et al.*, 2009; Chauhan *et al*., 2020). The small size of lipid particles can enhance drug release by increasing absorption through the stratum corneum. The particle size of NLC depends on the lipid matrix (lipid-surfactant) and the manufacturing method used. The composition of solid lipids and liquid lipids has a ratio of 70:30 to 99.9:0.1 with a total lipid composition of between 5-40%, while surfactants generally range from 0.5-5% (Souto *et al*., 2020; Garg *et al*., 2021). NLC generally uses a lipid matrix of waxes, fatty acids, ester glycerides, long-chain alcohols, and steroids *(*Beloqui *et al.,* 2016), while liquid lipids contain a mixture of mono-, di-, and triglycerides of different fatty acids with chain lengths and saturation (Elmowafy & Al-Sanea, 2021). In this research, the NLC system will be developed using vitamin D3 in various concentrations of 7:3, 8:2, and 9:1.

Methods

Material

The material used in this research were Vitamin D3 (Xi'an Prius Biological Engineering Co., Ltd), Monostearin (Rikevita SDN.BHD), Miglyol 812 (Sigma Aldrich-USA), Tween 20 (Industria Chimica Panzeri), PEG 400 (Vim Spectrum), Ethanol Pa (Merck).

Preparation of the NLC system

The method used was High Shear Hot-Homogenisation. The ratios used between solid lipids and liquid lipids are 7:3, 8:2, and 9:1. Vitamin D3 NLC system was prepared by melting the lipid phase (Monostearin and Miglyol 812) and vitamin D3 at 70°C at the same time. Surfactant solution (Tween 20 and phosphate buffer pH 6.0 ± 0.1) and cosurfactant solution (PEG 400 and phosphate buffer pH 6.0 ± 0.1) were prepared and heated at 70°C and dispersed into the hot lipid phase using ultra-turrax at the same temperature at 20,000 rpm for three by ten minutes. In the cooling stage, a magnetic stirrer was used at a speed of 500 rpm until it reached a temperature of 25°C. The NLC was then weighed to determine the final weight of the NLC.

Table I: Formulation of vitamin D3 NLC system

Number	Materials	Function	Concentration (%w/w)		
			F1	F2	F3
1	Vitamin D3	Active substance	1	1	1
2	Monostearin	Solid Lipid	7	8	9
3	Miglyol 812	Liquid Lipid	3	2	1
4	Tween 20	Surfactant	3	3	3
5	PEG 400	Co-suractant	2	2	2
6	Phosphate buffer pH 6.0	Aqueous phase	ad 100	ad 100	ad 100

Physicochemical characterisation of vitamin D3 NLC system

Fourier transform infrared spectroscopy

The Infrared spectra were examined using Fourier Transform Infrared (FTIR) spectroscopy. The purpose of this tool is to determine the chemical interactions between the active substances and other matrices during preparation. FTIR examination in this study was carried out on all materials made for the study and on the finished Vit D3 NLC system. An analytical method uses infrared light to identify and measure substances and predict their chemical structure. Energy at various infrared light frequencies is recorded and transmitted to the interferometer. The signal is converted into an interferogram, and mathematical calculations (the Fourier transform) for the signal will produce an identical spectrum in infrared spectroscopy. The sample is placed on a plate, and then the spectra are seen. Analysis using spectra results by comparing literature.

Visual observations

Testing was carried out by direct visualisation, including colour, clarity, homogeneity, and shape, to determine whether there was a physical change or not (Annisa *et al.,* 2016). Stable Nanostructured Lipid Carrier (NLC) was characterised by suitable consistency, a colour that matched the active ingredient, as well as a non-rancid and homogeneous odor.

pH

The pH was determined using a pH meter (Mettler Toledo, Seven Compact, Swiss) at 25°C. Measurements were made by inserting electrodes into the sample, and the results are indicated by the numbers displayed on the tool.

Viscosity

A viscosity examination was performed using a cone and plate viscometer (Cone & Plate, Brookfield DV3TLVCJ0, USA). This inspection aimed to determine the viscosity of the resulting NLC system. A sample of 0.5 – 2.0 mL was added to the sample cup, which was opened by the cover in the form of a stationary plate. The sample size depended on the cone used and must be air bubbles-free. Then, the sample cup was put back on the viscometer, turned on, and allowed to stand for a while until the reading stabilised.

Particle size and size distribution

Particles were analysed using the Delsa NanoTM (Beckman Coulter, USA) as a particle-size analyser tool. Particle size analysis by determining particle size by measuring the rate of fluctuation in the intensity of the laser light scattered by the particles as they diffuse through a liquid particle-size analyser tool. As much as 50 g of Vit D3 NLC system was added to 50 mL of distilled water and stirred at 500 rpm for ten minutes with a magnetic stirrer. The solution (2 mL) was added to 8 aquadest and stirred for two minutes at 100 rpm. About 2.0 mL of the sample was placed in the cuvette and placed in the sample holder. The data obtained are the average droplet diameter and polydispersity index.

Zeta potential

Zeta potential was checked using the Zetasizer tool (Anton Paar, Austria). Zeta potential is measured using the electrophoretic light scattering technique, where an electric field is applied to the sample, and the Doppler frequency shift of the scattered light is compared with a reference light beam to calculate the zeta potential. The sample was dispersed as much as 1 mL in 10 mL of distilled water until the concentration was obtained at the intensity of the instrument was optimum. The sample was then inserted into the sample holder, and the zeta potential was measured.

Entrapment efficiency of vitamin D3

Entrapment efficiency (EE) was a percentage measurement of entrapment carried out using a UV-Vis spectrophotometer. EE determines the percentage of active substances adsorbed in an NLC system. The measurement of entrapment efficiency was determined using the centrifugation method. Several samples (± 1.5 g) were centrifuged for 60 minutes at 3000 rpm. The supernatant was taken out, and 25 ml of ethanol was added. Then, one millilitre of solution is diluted with ethanol to 25 ml.

Results

Fourier transform infrared spectroscopy (FTIR)

The results of Vitamin D3 infrared spectra examination with NLC Formula 1, Formula 2, and Formula 3 can be seen in Figure 1. The infrared spectrum is a plot of absorption against intensity wavenumber expressed by the number of waves in units of cm^{-1} with a value range of 400-4000 cm^{-1}. Meanwhile, identifying shifts in bending is specific based on rocking in the 400-2000 cm^{-1} wave area. This area can be called the fingerprint area. The FTIR results produce the same spectrum pattern, and the peak transmittance of the wave number is similar. Vitamin D3 has specific peaks in the areas of 3300-3500 cm^{-1}, 2850-2970 cm^{-1}, and 1610-1680 cm-1; Monostearin, Miglyol 812, Tween 20, and PEG 400 have sharp peaks in the area of 2850–2970 cm^{-1}. The FTIR spectra of NLC F1, F2, and F3 have identical profiles and no new peaks when compared with their matrix. These results indicate no chemical interaction between vitamin D3 and the lipid matrix that can cause changes in functional groups that can produce new peaks in the FTIR spectra.

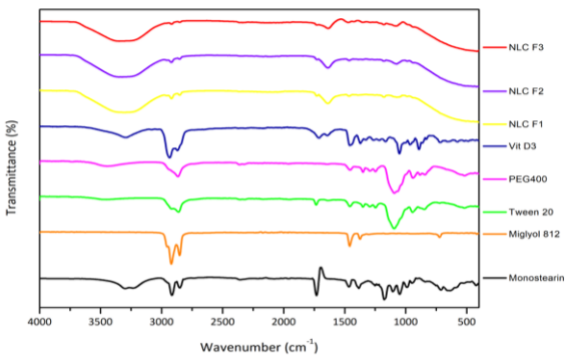

Figure 1: FTIR, Vit D3, F1, F2, F3

Visual observations

The visual observation test compared the results of the differences in solid and liquid lipid concentrations in the Vit D3 NLC system with ratios of 7:3, 8:2, and 9:1, as shown in Figure 2. The visualisation results showed that all Vit D3 NLC formulas were similar in that they had a yellowish colour, a typical lipid odour, and were semisolid.

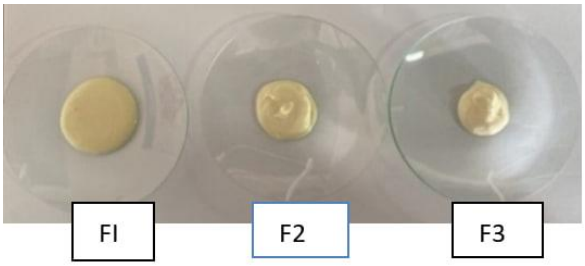

Figure 2: Visual observation test of NLC

pH

Parameters for measuring pH include the stable pH of the active substance and skin pH. pH measurement results are in the range of 5.4-5.8, as shown in Table II. The resulting pH value is near 6.0 because the NLC uses a phosphate buffer solution (pH 6.0) as a solvent. For topical delivery, the skin pH, which is 4.5-6.5, is used to provide comfort.

Table II: Physicochemical characterisation of NLC vitamin D3

Parameters	Formulation		
	FI (7:3)	F2 (8:2	F3 (9:1)
pH	5.4 ± 0.08	5.7 ± 0.11	5.8 ± 0.07
Viscosity (cp)	365.8 ± 20.51	433.1 ± 6.79	469.43 ± 7.59
Particle size (nm)	266.1 ± 60.03	337.5 ± 45.22	438.8 ± 7.51
Size distribution	0.38 ± 0.04	0.36 ± 0.05	0.42 ± 0.11
Zeta potential (mV)	-36.76 ± 1.4	-26,80 ± 2.38	-33.76 ± 2.23
Entrapment efficiency	5.4 ± 0.08	5.7 ± 0.11	5.8 ± 0.07

Viscosity test

The viscosity results of the NLC showed that formula 1 (7:3) had a value of 365.8 ± 20.51, formula 2 (8:2) had a value of 433.1 ± 6.79, and formula 3 (9:1) had a value of 469.43 ± 7.59. The Vit D3 NLC system, which has a ratio of solid lipid and liquid lipid F1 (7:3), provides the lowest viscosity. The higher the liquid lipid content, the lower the viscosity. Based on one-way ANOVA statistical analysis, it shows $p > 0.05$, which means there is no significant difference in all formulas.

Particle size and size distribution

Particle size resulted from a vitamin D3 NLC system of < 500 nm. Particle size inspection results in all formulas < 500nm. Particle sizes > 600nm tend to be in the stratum corneum and cannot penetrate deeper lipid layers (Danaei *et al.,* 2018). The particle size of the NLC system produced is F1 < F2 < F3. The average particle size is shown in Table II. The results of one-way ANOVA statistical analysis showed a significant difference in particle size ($p < 0.05$), and based on post-hoc with the least significant difference (LSD), there was a difference between F1 and F3, and F2 and F3. Particle size distribution is shown from the polydispersity index (PI). The PI value < 0.5 describes the homogeneity of the preparation. PI results from all formulas < 0.5 indicate that the Vit D3 NLC system was homogeneous.

Zeta potential

The results of measuring the zeta potential value of the vitamin D3 NLC system can be seen in Table II. The results showed that F2 was below the range of ± 30 mV. In general, dispersion systems with a zeta potential value out of range ±30mV have stable electrostatic properties (Khater *et al*., 2021). Based on Kruskal-Wallis's statistical analysis, all formulas have significant differences. The zeta potential value of the Vit D3 NLC system ranges from -26.80 mV to -36.76 mV.

Entrapment efficiency

Table II shows the results of measuring the Vit D3 NLC system entrapment efficiency (EE). The results show that increasing liquid lipid levels can increase the entrapment of Vit D3. Measurements of EE for all formulas obtained range from 79 to 82%. Based on statistical analysis using one-way ANOVA, there are no significant differences ($p > 0.05$) between F1, F2, and F3.

Discussion

The manufacturing method uses a high-shear homogeniser because the simple process has many advantages, such as ease of scale-up, no use of organic solvents, and shorter production time than other methods (Tamjidi *et al*., 2013). The formula was then evaluated against the vitamin D3 NLC system, which

included Fourier transform infrared (FTIR) examination. FTIR examination aims to observe chemical interactions between vitamin D3 preparations in formulas usually indicated by a shift or reduction in the peak of the drug function dropping or the formation of peaks that are different from the beginning. Transmission was measured from a wave number of 400-4000 cm^{-1}. The FTIR spectra from NLC F1, F2, and F3 have identical profiles because the constituent components and manufacturing methods are similar, differing only in the ratio of the amounts of liquid and solid lipids. When comparing the FTIR spectrum of the Vit D3 NLC system with the FTIR spectrum of the NLC matrix (Monostearin, Miglyol 812, Tween 20, and PEG 400), several changes in peak height represent the active substance embedded in the matrix. Overall, the resulting spectrum pattern is the same, and the peak transmittance of the wave number is also almost the same. These results indicated no chemical interaction between vitamin D3 and the lipid matrix, which can cause functional group changes and new peaks (Madni *et al*., 2015).

NLC was prepared using solid lipids (Monostearin) and liquid lipids (Miglyol 812) with various comparison ratios (F1 7:3, F2 8:2, F3 9:1). The solid lipids contained in the carrying system play a role in forming the lipid matrix core and providing stability to the system. The ratio of solid lipids, liquid lipids, and surfactants significantly affected the stabilities of the NLC. Moreover, adding a water phase (phosphate buffer) to the NLC system could improve the system's stability during the manufacturing process (Tamjidi *et al.*, 2013). The addition of surfactants to the concentration and composition of the formula affected the stability of NLC.

The composition of solid lipids used was greater than that of liquid lipids in the NLC formula, which was 7:3, 8:2, and 9:1. It greatly affected the characterisation results of the Vit D3 NLC system. Solid lipids, as the basic framework for forming NLC, determined the final characteristics of the NLC. The ratio of solid lipids to liquid lipids was 7:3. It produced good characterisation and met the physical and chemical quality of the Vit D3 NLC system. This was because the pH value was 5.4 ± 0.07, and the particle size was 266.1 ± 60.03nm, which tended to be small. The addition of liquid lipids to the formula played a role in reducing the particle size. Increasing the concentration of liquid lipids decreased the size of the NLC particles.

The pH measurement results of all formulas varied between 5.4 to 5.8. The average pH value for F1, F2, and F3 were 5.4 ± 0.08, 5.7 ± 0.11, and 5.8 ± 0.07 respectively. All preparation formulas have a pH close to neutral that matches the skin's pH balance (4.5-6.5) (Listiyana *et al*., 2020). Thus, if the pH is classified as suitable for use in topical NLC preparations. Based on the one-way ANOVA statistical analysis results, there was no significant difference from all NLC systems.

One of the characteristics important in the development of NLC systems is particle size. The smallest NLC particle size was found for F1. Particle sizes that tend to be small are well distributed, making the nanoparticle system more stable.

The viscosity value in the vitamin D3 NLC system ranged from 365.8 to 469.43cp. Viscosity describes the ease of a preparation to be smeared and predicts the ease of molecules to move drug release. If the preparation is too viscous, it will be difficult to defecate from the base and penetrate the skin. Semisolid preparations generally have a viscosity range of 32.5 – 24,495cps, and the Vitamin D3 NLC system is included in this preparation.

The results of the entrapment efficiency test showed that the average entrapment value was quite high for the three formulations. Statistically, using one-way ANOVA on particle size measurements, the significance was > 0.05. This indicated no significant differences between the entrapment efficiencies of all formulas. The entrapment efficiency values of F1, F2, and F3 increased by increasing the ratio of liquid lipids in the formulation. The average entrapment efficiency of F1, F2, and F3, respectively, was 82.18 ± 1.00, 81.29 ± 0.63, and 79.53 ± 0.07, and the results obtained from the F1 formulation showed that the highest entrapment effectiveness was 82.18%.

This is in line with the theory that increasing the liquid lipid ratio in the formula will increase the flexibility of the NLC core by affecting the imperfection of the crystal lattice so that when the lipid phase occurs, a lot of drug is trapped in the system (Apostolou *et al.*, 2021). This increase in the flexibility of the NLC core is useful for preventing expulsion. The ability of miglyol 812 to increase the solubility of Vitamin D3 causes an increase in entrapment efficiency. Miglyol 812 produces an imperfect crystal form, resulting in a larger space in the crystal, which leads to a greater drug-holding capacity (Annisa *et al*., 2016).

The lipophilic preparation includes an entrapment efficiency value of > 50%. The high entrapment efficiency value results from more solid lipids that function as adsorbent media, resulting in more drug load. The high efficiency value determines that the drug is more suitable to be classified as a topical preparation.

Conclusion

The vitamin D3 NLC system with different lipid concentration ratios affects the physical and chemical characteristics. In this study, increasing the liquid lipid concentration can reduce particle size and viscosity and increase entrapment efficiency.

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