STABILITY AND IN VITRO DRUG RELEASE STUDIES OF NIOSOME VITAMIN D3

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Abstract: This study aimed to determine the stability of niosome vitamin D3 at two different temperatures, room temperature 25oC and 4oC, and to view the in vitro release profile and determine the kinetics model of the release. Niosome is a drug delivery system formed from surfactants and cholesterol in the form of vesicles that entrap drugs in their matrix. This allows the drug trapped in the niosome matrix to be protected from the influence of the biological environment, which can reduce or eliminate drug activity. In addition, in the presence of a vesicle matrix system, it often provides a modified release. From a previous study conducted by Audia et al., vitamin D3 niosome were prepared from Span 60 and cholesterol using the thin layer method. In this study, a stability test was carried out for 30 days at two different storage temperatures, as well as an in vitro study of the release of vitamin D3 niosome to determine the release kinetics model. The results showed that vitamin D3 niosome were more stable at 4oC than at 25oC. The in vitro release test showed the release of vitamin D3 niosome at 3.25 ± 0.2% for 420 minutes with release kinetics following the Korsmeyer-Peppas kinetic model.

Keywords: release kinetics, niosome vitamin D3, Korsmeyer-Peppas

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INTRODUCTION

Nonionic surfactant and cholesterol vesicles, or what is known as niosome, is a form of drug delivery system which self assembly in aqueous media and produces a closed bilayer structure that is biologically acceptable [1–3]. This delivery system is starting to be widely used by researchers to formulate drugs because of its solubility, biocompatibility, and ability to be applied to hydrophilic and lipophilic materials. With their ability to entrap hydrophilic and lipophilic materials in their vesicle matrix, these materials can be protected from the influence of the biological environment in the body. They can control the release and prolong the activity of a drug [4]. In addition, the high stability and lower cost of making niosome compared to liposome make niosome more preferred [5]. Vitamin D3 is the main form vitamin D, has water-insoluble properties that can be obtained from food sources such as fish and eggs, and can also be produced in the human body. Vitamin D3 has many functions in the body. In addition to bone health, vitamin D3 affects cardiovascular, cancer, and immunity [6,7]. Unfortunately, some studies report that the stability of vitamin D3 is low. When exposed to light, air, or heat, vitamin D3 will be degraded and eventually lose its function [7]. In vitro drug release studies were conducted to determine the developed drug dosage form release kinetics model. It helps predict drug activity in vivo [8,9]. The methods commonly used in drug release studies for vesicle preparations are filtration, ultracentrifugation, extraction, and dialysis. These methods are based on separating the free and entrapped drugs in the vesicle matrix [8]. Linear regression models such as Higuchi and Korsmeyer-Peppas are the most frequently used kinetic models to describe non-linear diffusion profiles. The Korsmeyer-Peppas kinetic model is reported to be the best mathematical model to describe the kinetics of drug release from liposomes [8]. In this study, the mathematical model used to describe the kinetics of vitamin D3 release, as shown in Table 1, is zero-order, first-order, Higuchi and Korsmeyer-Peppas [9–12].

Table 1. Kinetic models applied for drug release study

Kinetic model	Equation
zero-order	$C_t = C_0 + k_0 t$
first-order	$\log C_0 - \log C_t = \frac{k_1 t}{2.303}$
Higuchi	$C_t = A[D(2C_0 - C_s)C_s.t]^2$
Korsmeyer-Peppas	$F = \frac{M_t}{M^m} = k \cdot t^n$

Ct s the concentration of drug released at time t, $C0$ is the initial concentration of drug released, $k0$ is a zero-order constant, $k1$ is a first-order constant, t is time in hours, A is the unit area, D is the diffusion coefficient, F is the fraction of drug released at time t , Mt adalah is the mass of drug released at time t, M is the total mass of drug in the preparation, km is the kinetic constant and n is the diffusion exponent [9 – 12].

MATERIAL AND METHODS

The material used in this study were vitamin D3 (Sigma-Aldrich), Span 60, cholesterol (Croda Europe Ltd., UK), methanol and ethanol (Sigma-Aldrich), PBS (Phosphate Buffer Saline), distilled water, and aqua Deion (PT. Ikapharmindo Putramas). And the instruments used were rotary evaporated (Stuart RE-300), bath sonicator (Branson), microcentrifuge (Dynamic Velocity 18R), particle size analyzer (Malvern), transmission electron microscopy (TEM), HPLC with a UV detector (L-2000 Hitachi, with L-2130 pump and Hitachi L-2420 UV-Vis detector).

Formulation of niosome vitamin D3

Based on research conducted by Audia et al., vitamin D3 niosome were prepared using a Span 60 surfactant and cholesterol. Niosome were prepared using the thin layer method by adding 10 mL of ethanol into a round bottom flask filled with Span 60, cholesterol, and vitamin D3. The round bottom flask was then placed on a rotary evaporator set at 60oC at a speed of 150 rpm for 30 minutes until the ethanol disappeared and a thin layer was formed on the walls of the flask. After being stored for 24 hours, the thin layer was then hydrated with 10 mL of aqua Deion until a complete dispersion was formed, then sonicated for 30 minutes [8]. The niosome were then characterized (morphology, particle size, zeta potential, and sorption efficiency) [13].

Stability studies of niosome vitamin D3

The stability test of niosome vitamin D3 was carried out at two temperatures, at room temperature of 25oC and 4oC, for a month. At intervals of 0, 7, 14, 21, and 30 days, samples were taken and analyzed for particle size, zeta potential, and entrapment efficiency [14,15].

In vitro release studies of niosome vitamin D3

The dialysis bag was soaked and rinsed with distilled water for 24 hours before use. Niosome dispersion of as much as 2 mL was put in a dialysis bag, and both ends were tied so the sample did not come out through the back. The bag was then put into 50 mL phosphate buffer pH 7.4 and stirred at 100 rpm at 37oC [7,14,16,17]. Dialysates were taken at intervals of 0, 60, 120, 180, 240, 300, 360, and 420 minutes. Each dialysate uptake was replaced with a phosphate buffer with the same uptake volume [3,7,17]. The samples were then analyzed using HPLC. In vitro release data were then analyzed to characterize and determine the drug release mechanism by fitting it with several kinetic models (Table 1) [12].

RESULTS AND DISCUSSION

Characteristics

Niosome of vitamin D3 made using the thin layer method by Audia et al. showed the character of Large Unilamellar Vesicles (LUV) niosome where the average particle size was 257.4 nm with a zeta potential of -49.1 mV, and entrapment efficiency of 94.081%. The morphology of the vitamin D3 niosome is shown in Figure-1.

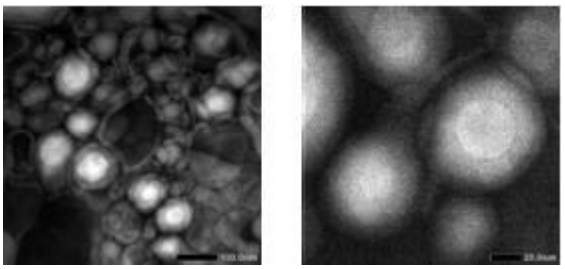

Figure-1. Morphology of niosom vitamin D3

Stability studies Particle size

The stability of the vitamin D3 niosomes is shown in Figure-2. The particle size of niosomes increased at room temperature storage, which was 269.5 nm on day 0 to 594.46 nm on day 30, while at 4oC, the particle size also increased, although not as big as the increase at room temperature, namely from 271.6 nm on day 0, to 365.76 nm on day 30. The increased particle size may be due to the surfactants' fusion or agromelization. At higher temperatures, the movement of molecules is faster

than at lower temperatures, and this causes the particle size to be larger [18].

Zeta potential

The zeta potential of niosome stored at room temperature on day 0 was -45.33 and decreased to -53.4 on day 30. Like storage at 4oC, the zeta potential decreased from -49.93 on day 0 to -54.06 on day 30. In general, charged niosome were better able to prevent aggregation and fusion than uncharged niosome [19].

Entrapment efficiency

In the stability test of vitamin D3 niosome, the entrapment efficiency of vitamin D3 niosome stored at 4oC on day 0 was 94.77% and increased to 96.65% on day 30. In contrast, the entrapment efficiency of niosome vitamin D3 at room temperature 25oC increased by 102.7% on day 30 from the previous 94.81% on day 0. The increase in entrapment efficiency may be due to the presence of free vitamin D3 trapped between the agglomerated particles [18].

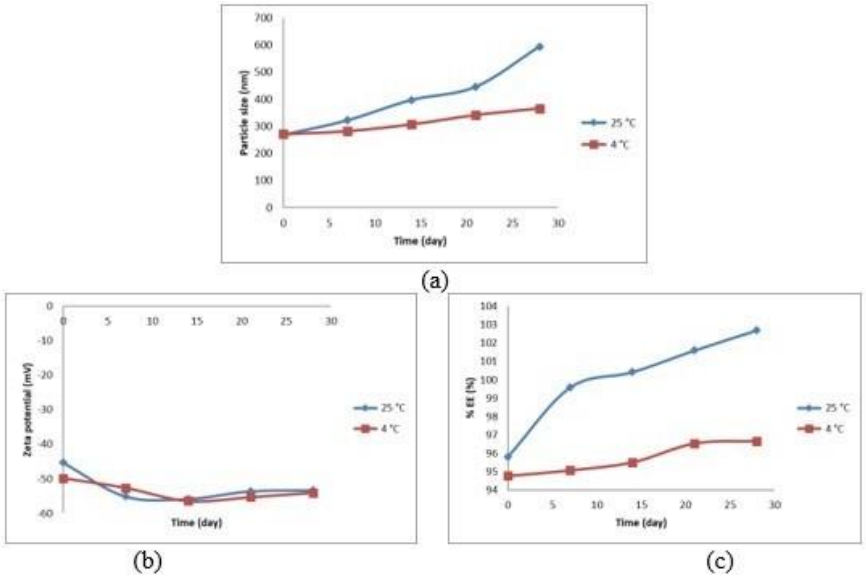

Figure-2. Stability studies of vitamin D3 on particle size (a), zeta potential (b), and entrapment efficiency (c).

In vitro release studies

The release profile of vitamin D3 can be seen in Figure-3. The results show that the cumulative amount of vitamin D3 released through the dialysis membrane until 420 minutes is 3.25%. The combination of surfactant and cholesterol can increase membrane rigidity and decrease permeability, especially cholesterol, which can change the fluidity of the bilayer chain, thereby reducing permeability [20]. As for the in vitro release data that was fitted to various release kinetics models to predict the mechanism of vitamin D3 release from niosome (Figur-4), it showed that vitamin D3 niosome were best explained by the kinetics of the Korsmeyer-Peppas model (Table 2), where the plot showed the highest linearity (R2 adjusted0.97 ± 0.009). The Korsmeyer-Peppas kinetic model, commonly called the anomalous release mechanism model, shows that drug release is controlled by more than one mechanism, diffusion, and erosion [12,21].

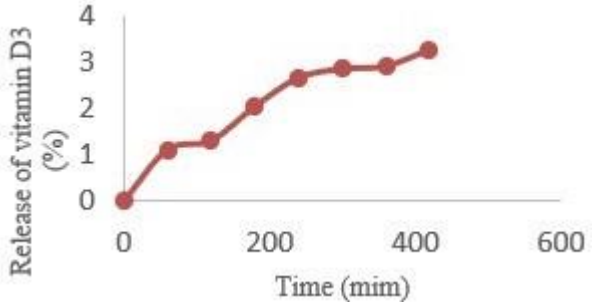

Figure-3. In vitro release profiles of niosom vitamin D3 in PBS pH 7,4

Table 2. Release kinetic models of niosome vitamin D3

Parameters	Kinetic models				
	Zero-orde	First-orde	Higuchi	Korsmeyer-Peppas	Hixson-Crowell
R^2 *adjusted*	0,856 ± 0,06	0,001	0,96 ± 0,02	0,97 ± 0,009	0,860 ± 0,06
AIC	3,361 ± 0,37	0,003 ± 0,003	-7,603 ± 4,49	-8,179 ± 2,37	3,145 ± 3,77
MSC	1,146 ± 0,57	0,001 ± 0,0005	2,516 ± 0,45	2,588 ± 0,2	1,173 ± 0,57

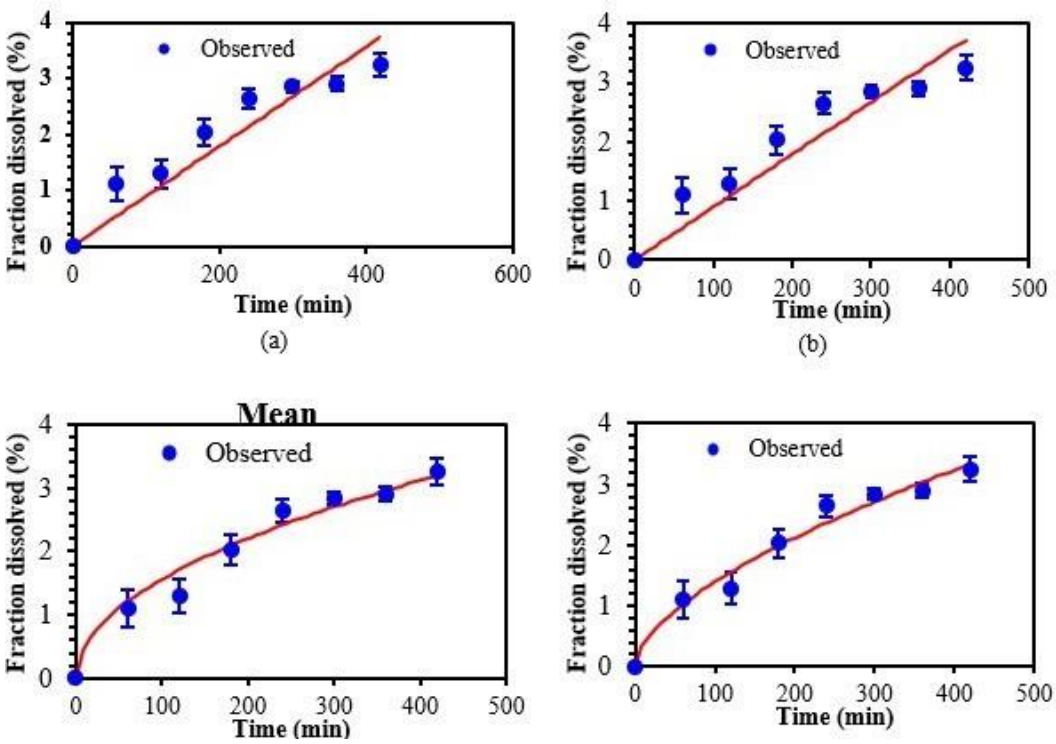

Figure-4. Release kinetics models profile of niosome vitamin D3

CONCLUSION

The results showed that the niosome of vitamin D3 was more stable at 4oC than at 25oC. The release of vitamin D3 niosome was 3.25 ± 0.2% for 420 minutes with release kinetics following the Korsmeyer-Peppas kinetic model.

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Ethical Approval

This study did not use experimental animals, so it does not require approval from the ethics committee.

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Conflict Of Interest

The authors declared that this research has no conflicts of interest.

Informed Consent

The research focused on stability and in vitro release study of niosome vitamin D3.

Authorship

Audia Triani Olii : researching and preparing manuscripts
Akhmad Kharis Nugroho : designed the research, prepared and revised the manuscript.
Ronny Martien : designed the research, prepared and revised the manuscript.
Sugeng Riyanto : designed the research, prepared and revised the manuscript.

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