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Development of nanostructured lipid carriers for the encapsulation and controlled release of vitamin D3

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ABSTRACT

Nanostructured lipid carriers (NLCs) for encapsulating vitamin D3 (VD3), a lipophilic vitamin, were successfully fabricated by hot high pressure homogenization. The physicochemical properties of the VD3-NLCs were characterized, and the release profiles of VD3 in simulated gastrointestinal fluids were investigated. Optimum VD3-NLCs were obtained with a small diameter (132.9 nm), a high zeta potential (−41.90 mV), and a high encapsulation efficiency (85.6%). The stability of the VD3-NLCs was tested during 20 days of storage at 25 °C under a wide range of pHs. *In vitro* digestion in simulated gastrointestinal fluids demonstrated their capability for controlled release because the NLCs were able to remain stable and protect the VD3 in simulated stomach fluid, but released more than 90% of the VD3 in simulated intestinal fluid. Therefore, the developed NLCs are promising carriers for increasing the oral bioavailability of VD3.

1. Introduction

Vitamin D (VD) is a lipid soluble vitamin that commonly occurs in two forms, namely vitamin D2 (VD2, ergocalciferol) and vitamin D3 (VD3, cholecalciferol) (Ziani, Fang, & McClements, 2012). VD2 is produced by UV irradiation of ergosterol in plants, whereas VD3 is synthesized in the human epidermis or found in oily fish and egg yolks (Lee, O'Keefe, Bell, Hensrud, & Holick, 2008). VD increases the intestinal absorption of calcium and promotes normal bone formation and mineralization (Gueli et al., 2012). Furthermore, VD acts as an antioxidant (Wiseman, 1993), maintains cardiovascular health, enhances the immune function, and exhibits anticancer effects (Gueli et al., 2012). Although both forms of VD exhibit similar antirachitic effects, VD3 is substantially more potent than VD2, and is thus preferred (Armas, Hollis, & Heaney, 2004).

VD deficiency is common, affecting 30–50% of the general population (Lee, O'Keefe, Bell, Hensrud, & Holick, 2008). VD deficiency is associated with an increased risk of cardiovascular disease and diabetes, inflammation, and rickets in children, and is caused by various factors, including an indoor lifestyle, poor diet, aging, obesity and malabsorption (Gueli et al., 2012; Lee, O'Keefe, Bell, Hensrud, & Holick, 2008). Thus, supplementation of VD is necessary to prevent deficiency and its associated effects on health. However, VD is easily degraded when exposed to light, air or heat, losing its functionality and health benefits, and also exhibits poor water solubility and oral bioavailability. Hence, encapsulation with a lipid delivery system is desirable to increase the stability, preserve the bioactivity, and enhance the absorption of this vitamin (Luo, Teng, & Wang, 2012; Ozturk, Argin, Ozilgen, & McClements, 2015; Sun et al., 2012).

Several methods have been applied for encapsulating VD3, including incorporating it in cyclodextrin (Delaurent, Siouffi, & Pèpe, 1998), zein nanoparticles coated with carboxymethyl chitosan (Luo et al., 2012), and micro and nanoemulsions (Ozturk et al., 2015; Ziani et al., 2012). Nevertheless, some disadvantages remain, such as poor formation, poor stability and fast release of emulsions (Ziani et al., 2012).

Solid lipid nanoparticles (SLNs) were developed in the 1990s as an alternative to nanoemulsions, and differ from them in that a solid lipid replaces the liquid lipid in nanoemulsions. However, even though they exhibit high biocompatibility and allow for prolonged release, their loading capacity tends to be low and the encapsulated compound tends to be expelled during storage (Shin, Kim, & Park, 2015). Hence, to overcome these disadvantages, nanostructured lipid carriers (NLCs) were developed (Müller, Radtke, & Wissing, 2002). As an alternative of SLNs, NLCs consist of solid lipid and liquid lipid at room temperature. In contrast to

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SLNs, the lipid droplets are partially crystallized with an amorphous structure and the oil is in the core of the carrier, resulting in higher loading capacity and controlled release of the encapsulated compound (Shin et al., 2015; Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013). Furthermore, the feasibility of mass production, nontoxicity, and availability of excipients make NLCs industrially viable (Aditya et al., 2014). Thus, NLCs can be considered as an advanced delivery system that can be valuable for encapsulating functional lipophilic compounds, such as fat-soluble vitamins.

The goal of this study was to develop NLCs to encapsulate VD3, aiming to improve its oral bioavailability. The NLCs were prepared by hot high pressure homogenization and their physicochemical properties were characterized. Several solid and liquid lipids were evaluated to select the best combination. The pH and storage stability of the fabricated NLCs was examined. Furthermore, to evaluate the suitability of the NLCs for oral delivery, their stability was evaluated in simulated gastric and intestinal fluids, and the release of VD3 from the carriers was determined *in vitro*.

2. Materials and methods

2.1. Materials

Vitamin D3 (VD3, cholecalciferol, 99% purity) was obtained from ALFA Chemicals (Seoul, Korea). Oleic acid (liquid lipid), glycerol monostearate (solid lipid), and Tween 80 (emulsifier) were obtained from Daejung Chemicals (Seoul, Korea). Lipases from porcine pancreas, bile extract porcine, pepsin from porcine gastric mucosa, and pancreatin from porcine pancreas were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and water were purchased from Merck (Darmstadt, Germany).

2.2. Liquid and solid lipid screening

VD3-loaded nanostructured lipid carriers (VD3-NLCs) were prepared using both solid and liquid lipids, selected according to their VD3 solubility and compatibility, respectively. The solubility of VD3 in oil was determined using a method similar to those used in microemulsions (Chaurasiya et al., 2012). Briefly, 10 mg of VD3 was added to each liquid lipid and heated at 80 °C in a water bath for 30 min. The resulting crystalline slurry was centrifuged at 4 °C and 10,000 rpm for 10 min, and then the supernatant was collected. The VD3 content in the supernatant was analyzed by HPLC. For the solid lipid screening, the VD3 and solid lipid were blended and heated at 80 °C in a water bath for 30 min, then the mixture was cooled down to room temperature. The mixture was partially solidified, and the remaining liquid was analyzed by HPLC to determine the VD3 content in the solid lipid.

2.3. HPLC analysis

A Shimadzu D-20A HPLC (Kyoto, Japan) fitted with a UV absorbance detector (operated at 265 nm) and an ACE5 C18 column (4.6 × 250 mm, 5 μm; Advanced Chromatography Technologies, Aberdeen, UK) was used to determine the amount of VD3 in the samples. The mobile phase was 100% methanol pumped at a flow rate of 1.0 mL/min. A sample volume of 20 μL was injected.

2.4. Fabrication of VD3-NLCs

VD3-NLCs were fabricated using hot high pressure homogenization as described in previous reports (Liedtke, Wissing, Müller, & Mäder, 2000; Mehnert & Mäder, 2001), with modifications. Briefly, an aqueous surfactant phase consisting of double distilled water and Tween 80 was prepared and heated at 80 °C, in a water bath, before adding it to the oil phase. Simultaneously, the lipid phase consisting of VD3 and solid and liquid lipids was prepared and heated up to 80 °C prior to mixing with the water phase. The hot aqueous phase was added to the lipid phase and homogenized using a high speed homogenizer (HG-15-A, Daihan Co., Seoul, Korea) at 10,000 rpm for 1 min. Subsequently, the mixture was subjected to high pressure homogenization (EmulsiFlex®-C3, Avestin, Inc., Ottawa, Canada; 0 to 10 cycles) at 10,000 psi. The resulting hot emulsion was cooled down to room temperature to form the VD3-NLCs.

2.5. Particle size, polydispersity index, and zeta potential

The mean particle size, size distribution, and zeta potential of the VD3-NLCs were measured by dynamic light scattering (DLS), using a zeta potential and particle size analyzer (ELSZ-1000, Otsuka Electronics Co., Ltd., Osaka, Japan) at a fixed detector angle of 90° and 25 °C. Samples were dispersed in distilled water (pH 6.5) in a ratio of 1:9 (NLCs: water). The particle size was expressed as mean diameter (Z-average) and the particle size distribution was expressed in terms of intensity (differential and cumulative,%). The sample was injected directly into the chamber of the ELSZ-1000 instrument, and the zeta potential of the particles was determined by measuring the direction and velocity of their droplet movement in a defined electric field (Laser Doppler Method).

2.6. Transmission electron microscopy

The morphology of the VD3-NLCs was determined by transmission electron microscopy (TEM; HT 7700, Hitachi, Tokyo, Japan). Imaging was performed at 100 kV and magnification ×80,000. A drop of sample was placed onto a carbon-coated copper grid (Ted Pella, Redding, CA, USA) and the excess was removed with filter paper. This step was repeated three times. Finally, the grid was stained with 1% (w/v) uranyl acetate for 3 min and dried overnight prior to image capture using TEM.

2.7. Encapsulation efficiency of VD3-NLCs

The encapsulation efficiency (EE) of the VD3-NLCs was determined by HPLC. The VD3-NLCs were stirred in hexane at 25 °C for 2 h and centrifuged at 10,000 rpm for 10 min. The obtained supernatant was mixed with methanol in a ratio of 1:9 (v/v) and analyzed by HPLC to determine the content of free VD3. EE was calculated using the following Eq. (1):

$$\text{Encapsulation efficiency } (\%) = \frac{VD_T - VD_F}{VD_T} \times 100 \quad (1)$$

where VD_T is the total amount of VD3 and VD_F is the amount of free VD3.

2.8. pH stability of VD3-NLCs

To assess the pH stability of the VD3-NLCs, their particle size, polydispersity index (PDI), zeta potential and EE were analyzed at room temperature under various pH conditions (2.0, 4.0, 7.0, and 10.0). Briefly, 1 mL of VD3-NLCs was dispersed in 9 mL of distilled water, and the pH was adjusted with NaOH and HCl. The samples were incubated for 2 h prior to analysis. The storage stability of the VD3-NLCs was assessed considering their changes in particle size, zeta potential, and PDI during various storage times (0–20 days) at 25 °C.

In addition, differential scanning calorimetry (DSC) of pure ingredients (GMS and VD3), blank NLCs, and VD3-NLCs was performed to determine their melting points. DSC analysis was performed using a Q-2000 thermal analyzer (TA Instruments Co.,