MEDS Clinical Medicine (2026)
Clausius Scientific Press, Canada

DOI: 10.23977/medsc.2026.070101
ISSN 2616-1907 Vol. 7 Num. 1

Evaluation of a Bile-Independent Vitamin D_3 Nano-Microencapsulated Delivery System: Efficacy of Amphiphilic Modified Starch in Overcoming Absorption Barriers and Storage Instability

Gloria Kuai[1,*], Doris Dai[1], Naizhuang Jake Wang[1], Amelia Wang[1], Mok Wai Fun[1]

[1]ABINOSA LIMITED, 1500 N GRANT ST, STE C, Denver, Co 80203, US
**Corresponding author: glorialyyyun@gmail.com*

Keywords: Vitamin D_3, Nano-microencapsulation, SEDDS, OSA starch, MCT

Abstract: This study aimed to evaluate a novel nano-microencapsulated self-emulsifying delivery system designed to overcome the dual challenges of storage instability and bile-dependent absorption of vitamin D_3. The system utilizes octenyl succinic anhydride (OSA)-modified starch as an amphiphilic wall material and medium-chain triglycerides (MCT) as the carrier to encapsulate vitamin D_3 into solid microcapsules. Performance was assessed through an accelerated stability test (40 ℃/75% RH, 6 months) and an in vitro dissolution study in bile-free simulated intestinal fluid. Results showed that the system maintained a vitamin D_3 retention rate of 98.4% after accelerated aging, significantly higher than the 61.4% retention of a conventional corn-oil control. In the bile-free medium, cumulative release reached 96.2% within 60 minutes, with a burst release of 88.5% in the first 15 minutes, and the system spontaneously formed uniform nanomicelles with an average particle size of 168.5 nm. In conclusion, the nano-microencapsulated system effectively enhanced the oxidative stability of vitamin D_3 through solid-state encapsulation and achieved efficient bile-independent release and absorption via self-emulsification. This offers a promising new strategy for vitamin D_3 delivery in populations with compromised digestive function.

1. Introduction

1.1 Physiological Importance and Global Deficiency

Cholecalciferol (Vitamin D_3) is a pleiotropic secosteroid hormone precursor essential for maintaining physiological homeostasis. While classically defined by its role in calcium-phosphorus metabolism and skeletal integrity, modern research has identified Vitamin D Receptors (VDR) across a wide range of tissues, implicating the vitamin in immune modulation, cardiovascular health, and neuroprotection[1]. Despite its biological importance, vitamin D deficiency remains a “silent epidemic,” affecting approximately 50% of the global population. Although dietary supplements are the primary mitigation strategy, the therapeutic potential of oral vitamin D_3 is often limited by two fundamental physicochemical barriers: ex vivo instability during storage and in vivo absorption variability.

1.2 The Stability Challenge: Oxidative Sensitivity

Structurally, Vitamin D_3 contains a conjugated cis-triene system that renders it thermodynamically unstable. The molecule is highly sensitive to environmental stressors, particularly UV radiation, heat, and atmospheric oxygen. Upon exposure, Vitamin D_3 undergoes oxidative cleavage or isomerization into inactive byproducts such as trans-vitamin D_3 or isotachysterol[1]. Standard delivery forms, such as oil-based softgels, often fail to provide an adequate oxygen barrier. Studies indicate that unsaturated fatty acids in vegetable oils can undergo lipid peroxidation, propagating free radicals that attack Vitamin D_3. Under accelerated conditions, potency losses of 40-60% have been reported [2]. To counteract this, manufacturers often add significant "overages" (excess active ingredient), raising costs and potential safety concerns regarding hypervitaminosis.

1.3 The Bioavailability Challenge: Bile Dependency

A more complex clinical barrier is the physiological limit on absorption. Vitamin D_3 is highly lipophilic, making it practically insoluble in the aqueous environment of the gastrointestinal tract. Absorption is rate-limited by diffusion across the Unstirred Water Layer (UWL) of the intestinal epithelium. Under normal physiology, the body secretes bile salts to emulsify dietary fats into mixed micelles, which ferry lipophilic nutrients across the UWL[3]. Consequently, Vitamin D_3 bioavailability is “bile-dependent.” This poses a therapeutic disadvantage for populations with compromised bile synthesis or secretion, such as the elderly, patients with cholestatic liver disease, or those with cystic fibrosis[4]. In these "bile-deficient" scenarios, standard lipid formulations fail to solubilize, resulting in negligible absorption.

1.4 Technological Innovation: SEDDS and Microencapsulation

To address these dual challenges, research has focused on Self-Emulsifying Drug Delivery Systems (SEDDS) combined with microencapsulation, as shown in **Fig.1**. An ideal SEDDS formulation spontaneously forms fine oil-in-water (O/W) emulsions upon contact with gastrointestinal fluids, creating a “pre-digested” state independent of physiological bile[5]. This study evaluates a system integrating two synergistic technologies:

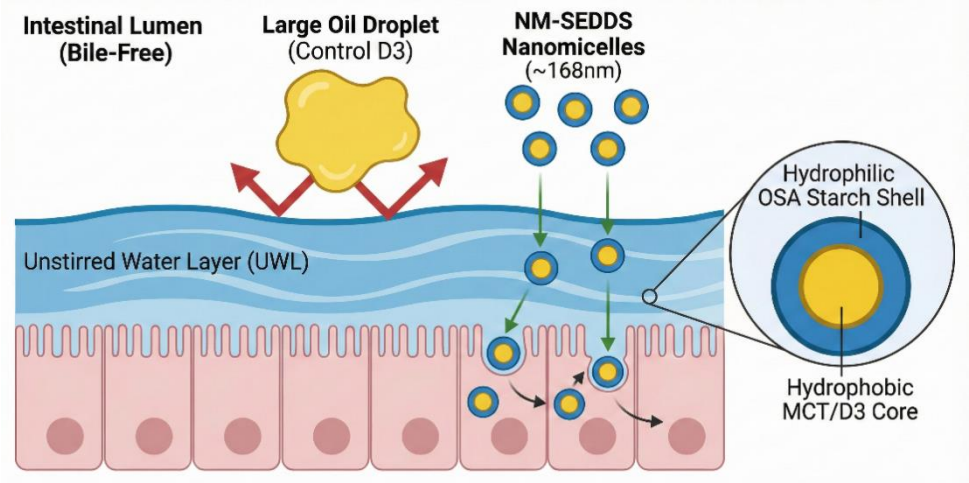

Figure 1. Design and Working Principle of the Bile-Independent Vitamin D_3 Nano-Microencapsulated Delivery System.

1) **Medium Chain Triglycerides (MCT) as Carrier:** Unlike Long Chain Triglycerides (LCT), MCTs are absorbed directly into the portal circulation, bypassing the lymphatic system and

offering kinetic advantages[6].

2) **Sodium Octenyl Succinate (OSA) Starch:** A chemically modified polysaccharide with amphiphilic properties. The hydrophobic octenyl succinate groups anchor into the oil phase, while the hydrophilic starch backbone stabilizes the interface, forming a robust barrier against oxygen and coalescence[7].

1.5 Study Objectives

The primary objective of this study is to evaluate the physicochemical performance of this OSA Starch-MCT Vitamin D_3 system. Specifically, we aim to validate:

Thermodynamic Stability: That the solid-state microencapsulation significantly outperforms standard oil formulations in retaining potency under accelerated aging (40 ℃/75% RH).

Bile-Independent Bioavailability: That the system spontaneously releases and emulsifies Vitamin D_3 in bile-free simulated intestinal fluid, generating uniform nanomicelles to overcome physiological absorption bottlenecks.

2. Materials and Methods

2.1 Materials and Reagents

Test formulation (NM-SEDDS): A free-flowing nano-microencapsulated powder containing cholecalciferol dissolved in MCT oil (coconut source), encapsulated within an OSA-starch matrix. The core-to-wall ratio was optimized to ensure complete encapsulation[8].

Control formulation: Crystalline cholecalciferol dissolved in pharmaceutical-grade corn oil, simulating standard softgel contents.

Reagents: HPLC-grade methanol and acetonitrile (Sigma-Aldrich). Simulated intestinal fluid (SIF) reagents: monopotassium phosphate and sodium hydroxide (NaOH). Bile salts and lecithin were intentionally excluded from the SIF to create a “bile-deficient model.”

2.2 Preparation of Nano-Microcapsules

Vitamin D_3 was dissolved in MCT oil. This oil phase was emulsified into an aqueous solution of OSA starch using high-shear mixing followed by high-pressure homogenization to form a nanoemulsion. The emulsion was subsequently spray-dried (Inlet temp: 170-180 ℃) to produce the solid microcapsules[9].

2.3 Accelerated Stability Study via RP-HPLC

Stability testing followed ICH Q1A (R2) guidelines.

Conditions: Samples were sealed in HDPE bottles and stored at 40 ℃ ±2 ℃ and 75% ±5% RH.

Sampling: Analysis occurred at 0, 1, 3, and 6 months.

Quantification: Vitamin D_3 content was determined via Reverse-Phase HPLC (Agilent 1260), using a C18 column, Methanol: Acetonitrile mobile phase, and UV detection at 265 nm[2].

2.4 Bile-Independent in Vitro Dissolution

Dissolution kinetics were assessed using a modified USP Apparatus II (Paddle Method).

Medium: 900 mL Modified SIF (pH 6.8), without bile salts/phospholipids.

Settings: 37 \pm 0.5^\circ C, 75 rpm.

Sampling: Aliquots were withdrawn at 5, 15, 30, and 60 minutes and filtered (0.45 μm PTFE) to

remove non-micellized oil droplets[10].

Characterization: The particle size of the dispersion at 60 minutes was analyzed using Dynamic Light Scattering (DLS) to determine Z-Average diameter and Polydispersity Index (PDI)[11].

2.5 Statistical Analysis

All experiments were performed in triplicate (n=3). Data are presented as Mean ± Standard Deviation (SD). Differences were evaluated using t-tests, with significance defined as $p < 0.05$.

3. Results

3.1 Thermodynamic Stability Profile

Under accelerated aging conditions (40 ℃ / 75% RH), the control and test formulations exhibited drastically different degradation profiles. The oxidative stress of the environment rapidly compromised the standard oil-based Vitamin D_3.

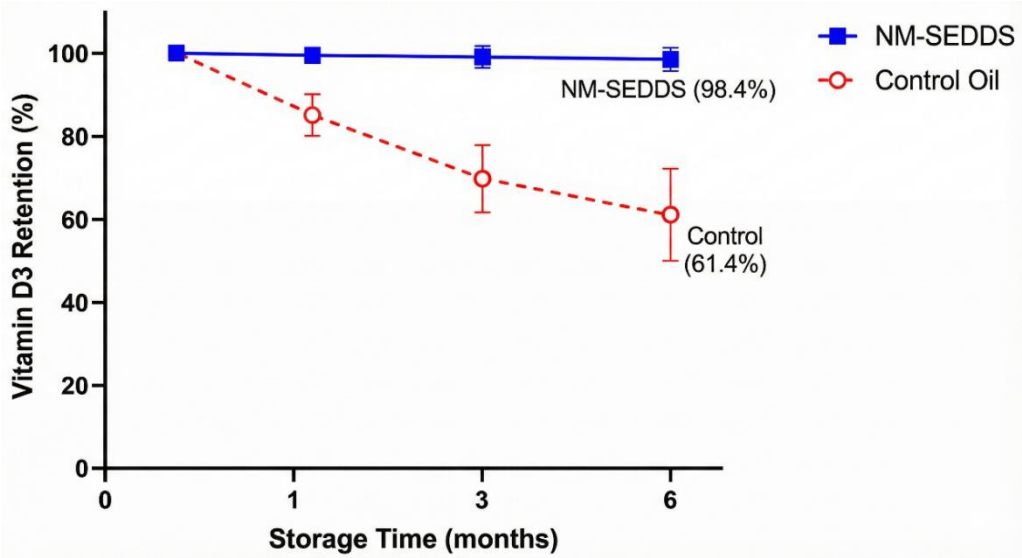

Figure 2. Comparative Retention Rates of Vitamin D3 under accelerated Conditions (40 ℃/75% RH).

As shown in **Fig.2**, the control formulation followed pseudo-first-order degradation kinetics ($R^2 > 0.98$), resulting in a significant potency loss of 38.6% by Month 6 ($p < 0.001$). This aligns with literature citing lipid peroxidation as a catalyst for Vitamin D_3 isomerization. In contrast, the NM-SEDDS formulation maintained 98.4% retention, demonstrating that the solid OSA starch matrix effectively immobilized the active ingredient and provided an impermeable barrier against oxygen.

3.2 Dissolution Kinetics in Bile-Free Environment

Fig.3 demonstrated that the dissolution test in bile-free media highlighted the functional disparity between passive oil carriers and active self-emulsifying systems.

Control Group: Consistent with its lipophilic nature, the standard oil formulation failed to disperse. Oil droplets floated on the surface, unable to pass the 0.45 μm filter. The cumulative release at 60 minutes was negligible at 2.8% ±0.5%.

Test Group (NM-SEDDS): The formulation exhibited "burst release" kinetics. Upon hydration, the OSA starch shell dissolved, releasing pre-formed nanodroplets.

- **15 Minutes: 88.5% ±2.1%** release.
- **60 Minutes: 96.2% ±1.4%** release.

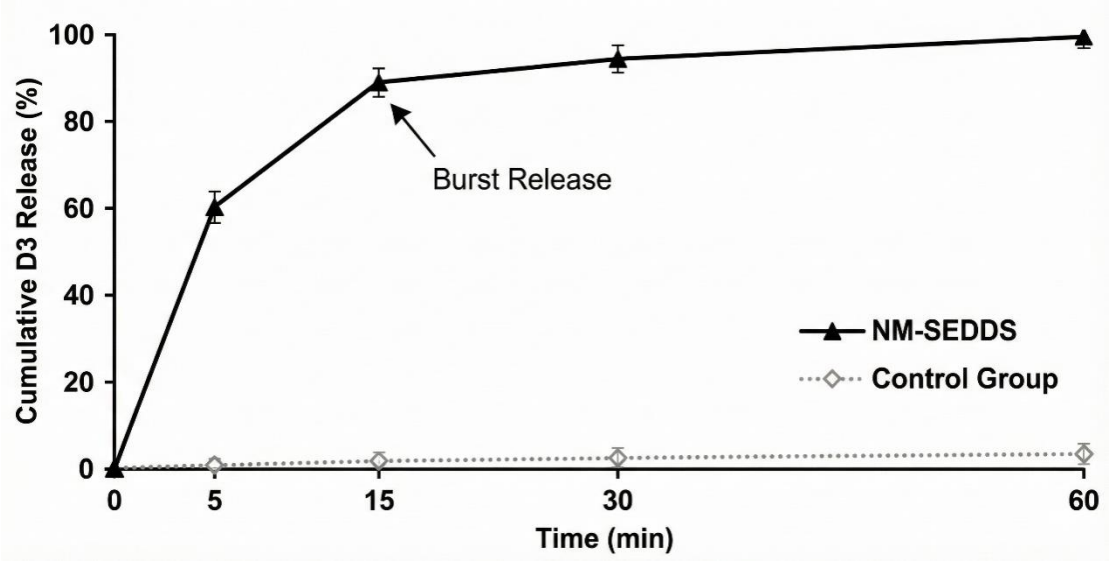

Figure 3. Dissolution Profile of Two Different Vitamin D3 Formulations.

3.3 Micellar Microstructure Characterization

Fig.4 indicated that Dynamic Light Scattering (DLS) analysis of the Test Group dispersion at 60 minutes confirmed the spontaneous formation of nanoscale structures.

Z-Average Diameter: 168.5 ± 4.2 nm. This size is optimal for intestinal transport, as particles <200 nm penetrate the mucus layer more efficiently.

Polydispersity Index (PDI): 0.18 ± 0.03. A PDI value below 0.2 indicates a narrow, monodisperse size distribution, suggesting a stable emulsion resistant to Ostwald ripening[11].

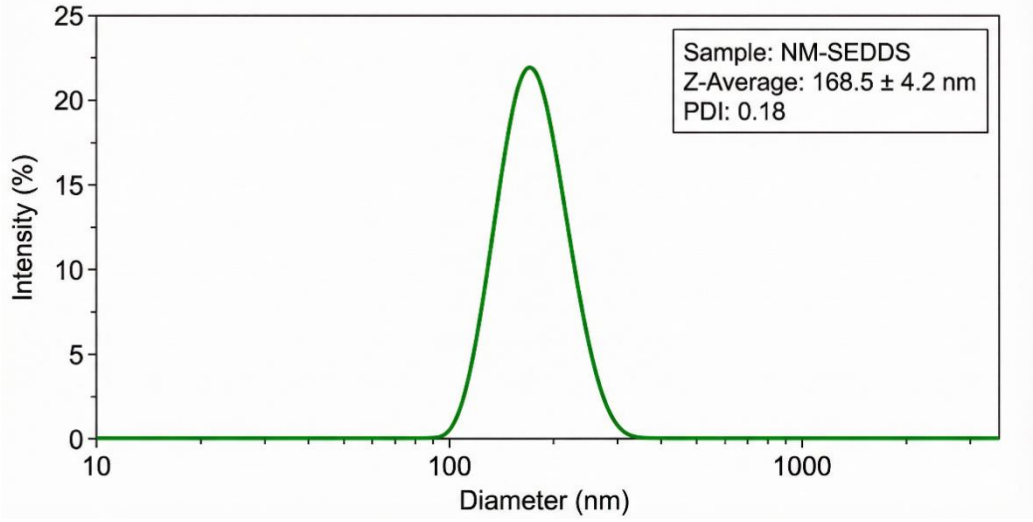

Figure 4. Particle Size Distribution of NM-SEDDS by Intensity.

4. Discussion

4.1 Mechanism of Enhanced Stability: Solid-State Immobilization

The dramatic difference in retention (98.4% vs. 61.4%) confirms that converting liquid vitamin D_3

into a solid-state microcapsule is a superior preservation strategy. In the liquid control, molecular mobility facilitates interactions between vitamin D_3 and dissolved oxygen or lipid peroxides. The OSA-starch matrix creates a “glassy” state that restricts molecular motion. Furthermore, OSA starch forms a dense interfacial film that acts as a hermetic seal, shielding the sensitive cis-triene structure from oxidative attack. This approach allows for a reduction in manufacturing overages, ensuring safer and more consistent dosing over the product’s shelf life.

4.2 The "Artificial Bile" Effect: Decoupling Absorption from Physiology

The most clinically relevant finding is the system’s performance in bile-free fluid. Physiologically, vitamin D_3 absorption is “gated” by the secretion of bile salts required to reach the critical micelle concentration (CMC). The NM-SEDDS bypasses this requirement. The amphiphilic octenyl succinate groups on the starch backbone function as “artificial bile,” lowering interfacial tension and stabilizing oil droplets through steric hindrance and electrostatic repulsion[12]. By generating 168 nm micelles ex vivo (in the stomach/intestinal lumen), the formulation presents the intestine with a “pre-digested” nutrient, theoretically enabling efficient absorption even in patients with cholestasis, cystic fibrosis, or age-related declines in digestive function.

4.3 Synergistic Role of MCTs

The use of MCTs provides a secondary kinetic advantage. Unlike Long Chain Triglycerides (LCT) found in corn oil, which require complex lymphatic processing, MCTs are rapidly hydrolyzed and absorbed via the portal vein. The combination of OSA starch (for emulsification) and MCT (for absorption kinetics) creates a dual-mechanism delivery system.

4.4 Clinical Implications

The ability to achieve >90% dissolution without bile salts suggests this formulation could reduce the high inter-individual variability seen in Vitamin D_3 supplementation. It offers a viable solution for patients who are "non-responders" to standard therapy due to malabsorption issues.

5. Conclusion

This study evaluated a nano-microencapsulated self-emulsifying delivery system for vitamin D_3. By leveraging the amphiphilic properties of OSA - modified starch and the bioavailability benefits of MCTs, the system successfully overcame the two major limitations of cholecalciferol supplementation. The findings revealed that, in its solid-state form, the system retained 98.4% of its potency after six months of accelerated aging, significantly surpassing the 61.4% retention observed in the oil-based control group. Furthermore, the system demonstrated spontaneous emulsification in bile-free media, achieving a release rate of 96.2% and forming uniform nanomicelles with a size of 168.5 nm, thereby effectively simulating physiological digestion without the need for endogenous bile. The final assessment indicates that this technology transforms vitamin D_3 from a passive, digestion-dependent nutrient into an active, self-emulsifying therapeutic agent, promising consistent and reliable delivery across diverse patient populations.

References

[1] Bikle, D. D. (2012). Vitamin D and the skin: physiology and pathophysiology. Reviews in Endocrine and Metabolic Disorders, 13(1), 3–19.
[2] Cui, A., Zhang T., Xiao P., et al. (2023). Global and regional prevalence of vitamin D deficiency in population-based

studies from 2000 to 2022: A pooled analysis of 7.9 million participants. Frontiers in Nutrition, 10, 1070808.
[3] Gidey T., Bekele T., Getachew P. (2025). Retention of Vitamins A and D_3 in Fortified Soybean Oil During Ethiopian Traditional Cooking and Shelf-Life Stability During Storage. Food Science & Nutrition, 13(9): e70843.
[4] Zareie, M., Abbasi A., Faghih S. (2021). Influence of Storage Conditions on the Stability of Vitamin D_3 in Fortified Canola Oil. Journal of Food Quality, 2021, 5599140.
[5] Rautureau, M., & Rambaud, J. C. (1981). Aqueous solubilisation of vitamin D_3 in normal man. Gut, 22(5), 393-397.
[6] Silva, M.C., et al. (2021). Vitamin D: One of the Key Ingredients in the Treatment of Malabsorption Syndromes? Metabolites, 11(4), 255.
[7] Uttreja P., Karnik I., Youssef A. A. A. et al. (2025). Self-Emulsifying Drug Delivery Systems (SEDDS): Transition from Liquid to Solid—A Comprehensive Review of Formulation, Characterization, Applications, and Future Trends. Pharmaceutics, 17(1), 63.
[8] Shah, N. D., et al. (2022). Effects of Wall Material on Medium-Chain Triglyceride (MCT) Oil Microcapsules Prepared by Spray Drying. Pharmaceutics, 14(6), 1281.
[9] Sweedman, M. C., Tizzotti M. J., Schäfer C., et al. (2013). Structure and physicochemical properties of octenyl succinic anhydride modified starches: A review. Carbohydrate Polymers, 92(1), 905-920.
[10] Matos M., Marefati A., Gutiérrez G., et al. (2016). Comparative Emulsifying Properties of Octenyl Succinic Anhydride (OSA)-Modified Starch: Granular Form vs Dissolved State. PLOS ONE, 11(8): e0160140.
[11] Seema R. Bajaj, Sandesh J. Marathe, Rekha S. Singhal (2021). Co-encapsulation of vitamins B12 and D3 using spray drying: Wall material optimization, product characterization, and release kinetics. Food Chemistry, 335, 127642.
[12] Gonnet, M., Lethuaut L., Boury F. (2010). New trends in encapsulation of liposoluble vitamins. Journal of Controlled Release, 146(3), 276-290.