Chapter 11

Nanoparticles for Delivery of Vitamin D: Challenges and Opportunities

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Additional information is available at the end of the chapter

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Abstract

In addition to the traditional role of calcium homeostasis and bone mineralization, calcitriol, the active metabolite of vitamin D, also displays other metabolic activities as antiproliferative, pro-differentiating, anti-inflammatory, immunomodulatory, and antineoplastic effects. Thus, the awareness that vitamin D insufficiency/deficiency may be associated with various diseases has grown. Also nowadays, vitamin D is recognized as a potential therapeutic agent in anticancer therapy. However, its administration presents some drawbacks such as high toxicity and low bioavailability. Thus, the use of nanotechnology may overcome these problems associated with vitamin D administration, allowing to decrease its toxicity in healthy tissues and increasing its bioavailability. In this chapter, an overview on vitamin D and its metabolic activity is presented, as well as a review of nanosystems for the encapsulation of vitamin D for different applications, such as food and pharmaceutical industries.

Keywords: vitamin D, calcitriol, nanotechnology, drug-delivery systems, nanoparticles

1. Introduction

Vitamin D (VD) was firstly identified as a vitamin and now is recognized as a prohormone. VD is a precursor to its active and biologically functional metabolite, a lipophilic seco-steroid hormone known as calcitriol [1].

In the epidermis, VD is produced in the form of cholecalciferol due to the action of sunlight [2]. Once produced, VD is translocated into the bloodstream. However, VD does not remain in

circulation for a long time as it is almost instantly stored on the adipose tissue or metabolized in the liver. As cholecalciferol is inert, it must be metabolized in the liver and the kidney through two hydroxylation processes to be converted to its active form, calcitriol [3]. Due to their structural similarities, calcitriol acts like classical steroid hormones binding to the vitamin D receptor (VDR) regulating target gene expression via both genomic and nongenomic pathways [4].

Despite its well-known regulation of calcium homeostasis and bone mineralization functions [5, 6], in the late 1970s VD was found in tissues not previously considered targets of VD action, which came to disclose that this hormone may carry out several other functions [3]. Calcitriol is nowadays associated with many additional actions including antiproliferative, pro-differentiating, anti-inflammatory, and immunomodulatory effects. For example, this hormone has the ability to suppress prostaglandin actions and enhance pro-inflammatory cytokines production, displaying a role in ceasing inflammatory process [7, 8]. Also, several studies support that VD may play a major role in tumor's pathogenesis, progression, and therapy [8–11].

Still, vitamin D deficiency is a worldwide well-recognized problem with health consequences. Due to the very limited dietary sources of VD and insufficient exposure to sunlight in northern regions, between 30 and 60% of the European and North American population suffer from VD deficiency [12]. Hence, formerly in western diets VD was added to food and beverage products such as milk, soft drinks, and bread, to increase its nutritional value. However, the acknowledgment of VD's high toxicity associated with the hypercalcemia phenomena [9, 13] and low bioavailability, since more than 75% of VD intake is catabolized and excreted before being converted to its active form or before its storage [2, 14], raised several issues to its administration resulting in the forbiddance of food fortification with VD.

However, the recognition that vitamin D deficiency as a health risk leads to the development of new functional foods and therapies using nanotechnologies for VD incorporation into foods and pharmaceutical formulations without reducing its bioavailability or activity. Thus, this chapter is dedicated to provide a systematic overview of VD and its activity, as nanocarriers for the delivery of VD.

2. Vitamin D and nanotechnology

2.1. Vitamin D: an overview

Vitamin D cannot technically be considered a vitamin in its true meaning. More than a micronutrient, vitamin D is a precursor to its active form, calcitriol. The latter is a lipophilic seco-steroid hormone [10]. Vitamin D is derived from a steroid precursor, a cholesterol-like molecule. A seco-steroid molecule is very similar to a steroid, but with a few differences in its skeleton. Whereas a steroid molecule core is constituted by 20 carbon atoms assembled in four fused rings (A–D), three cyclohexane rings (A, B and C) and one cyclopentane ring (D), the seco-steroid has its B ring broken [15, 16]. **Figure 1** shows the difference between these two

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types of molecules. In more recent years, it was revealed that vitamin D is not responsible for all biological activities linked with it, but actually it only represents a precursor to its active and biologically functional metabolite, known as calcitriol [5, 11]. Calcitriol is indeed the one, which displays several biologic activities formerly thought as vitamin D responsibility. Due to their similarities, calcitriol acts like classical steroid hormones. It binds to the vitamin D receptor regulating target gene expression via both genomic and nongenomic pathways [10].

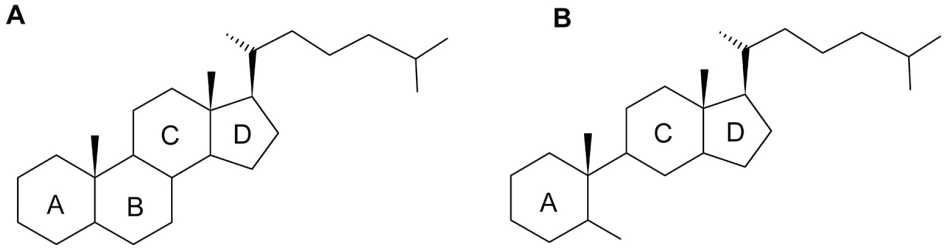

Figure 1. General chemical structures of (A) steroid and (B) seco-steroid (drawn in ACD/ChemSketch®).

In humans' bloodstream, vitamin D displays two main chemical forms: D2 or ergocalciferol, and D3 or cholecalciferol. The first one comes from the dietary source and can be found in some kinds of food such as salmon and cereals. On the other hand, the latter is produced in the epidermis from the action of sunlight and represents 95% of total blood's vitamin D [2]. These two forms exhibit chemical differences in their side chains (**Figure 2**). D2 has an extra methyl group at C24 and an extra double bond between C22 and C23.

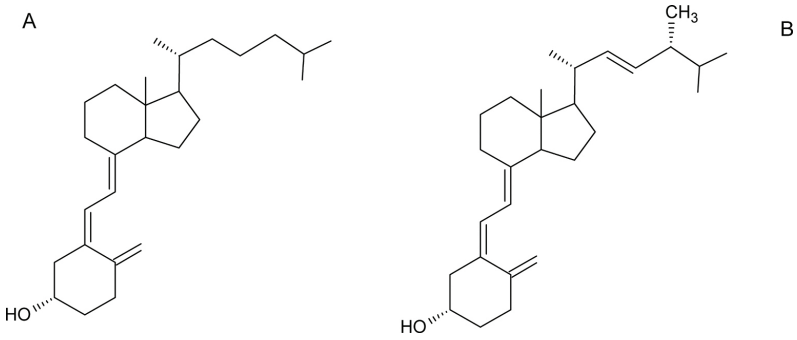

Figure 2. Chemical structures of (A) cholecalciferol and (B) ergocalciferol (drawn in ACD/ChemSketch®).

These structural changes, between D2 and D3, are reflected in their affinity for the carrier known as vitamin D-binding protein (DBP). Despite their metabolites' biologic activity being comparable, the fact that vitamin D3 has a higher affinity for DBP leads to the observation that in humans vitamin D3 potency is three times higher than vitamin D2's [15–17].

Due to this, the amount of work and research involving vitamin D3 is far superior, and all evidences reported to date on the efficacy of vitamin D for food fortification and the prevention of cancer and other diseases have been based on vitamin D3 [18]. For that reason only vitamin D3 will be covered for the next sections of this chapter.

2.2. Vitamin D metabolism

To better explain the process mentioned above, it is important to lay out the reaction steps involved in vitamin D synthesis. 7-Dehydrocholesterol is the precursor of pre-vitamin D. During exposure to the sunlight, UV radiation breaks the B ring of the precursor to form pre-vitamin D. Pre-D is rapidly isomerized by the body temperature into vitamin D (cholecalciferol), as it is schematized in **Figure 3** [11, 15, 19]. Once formed, cholecalciferol is translocated from the plasma membrane into the bloodstream where it bounds to DBP [19]. However, vitamin D does not remain in circulation for a long time as it is almost instantly stored on the adipose tissue or metabolized in the liver [3].

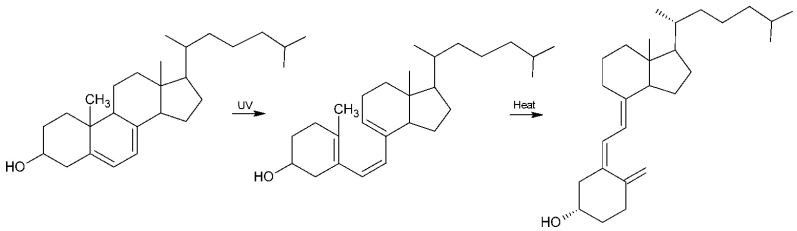

Figure 3. Image illustrating the production of cholecalciferol in epidermis (drawn in ACD/ChemSketch®).

Depending on its degree of hydroxylation, cholecalciferol can be found with three different chemical structures: calciol, calcidiol, and calcitriol. The first in order of appearance in the sequence of metabolic pathways, calciol, is the unhydroxylated and inactive form. Calcidiol is the monohydroxylated [16] and is the major blood circulating form [19] at concentrations in the range of 10–40 ng/ml [3]. Calcitriol is the dihydroxylated and active form responsible for all the vitamin D known biological actions [16]. These three different molecules can be compared in **Figure 4**.

Summarizing, cholecalciferol is inert and must be metabolized in the liver and the kidney through two hydroxylation processes to be converted to its active form. Thus, the first step of this activation, the hepatic 25-hydroxylation, inserts a hydroxyl group in C25 of cholecalciferol, thereby creating 25-hydroxyvitamin D3 (25-OH-D3). This step of 25-hydroxylation is mediated by a 25-hydroxylase enzyme (CYP2R1). Later in the kidney, 25-hydroxyvitamin D3 1α-hydroxylase (CYP27B1) enzyme is responsible for the insertion of one more OH group into the C1 of the A ring, converting it into calcitriol [3, 15, 20, 21]. In **Figure 4** also, the overall process of vitamin D activation is schematized.

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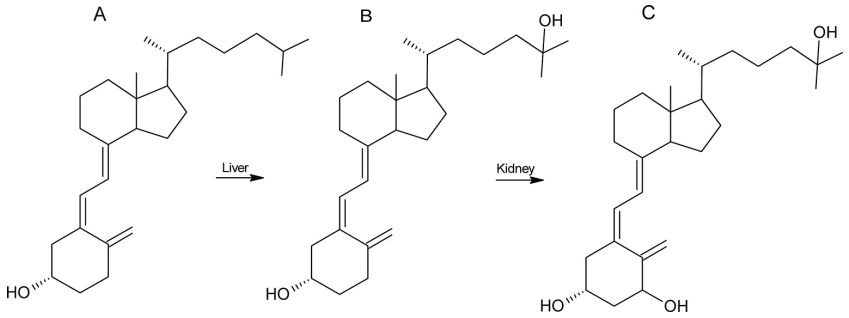

Figure 4. Representation of cholecalciferol three different chemical structures: (A) calcio, (B) calcidiol, and (C) calcitriol. This scheme also represents the overall chemical reactions involved in vitamin D activation (drawn in ACD/ChemSketch®).

Uncontrolled levels of calcitriol in the bloodstream may subsequently result in hypercalcemia phenomena [3, 11], related to a high risk of calcification of soft tissues especially intestine, kidney, and heart leading to organ failure and even death. As a result, the human body has a control mechanism that allows the inactivation of calcitriol. As this process of inactivation intends, the prevention of hypercalcemia therefore is upregulated by the administration of vitamin D, high levels of calcitriol itself, and high levels of serum calcium [3]. Hence, 24-hydroxylase enzyme (CYP24) inactivates calcitriol by hydroxylation (**Figure 5**). This reaction may occur in the liver or in any other target tissue, such as bone or intestine. The obtained inactive form, calcitroic acid, is metabolized and excreted. As the product is 10 times less biologically active than calcitriol, it has low affinity for VDR. For this reason, it is the main biliary excretory of vitamin D, since it is easily eliminated [3, 11].

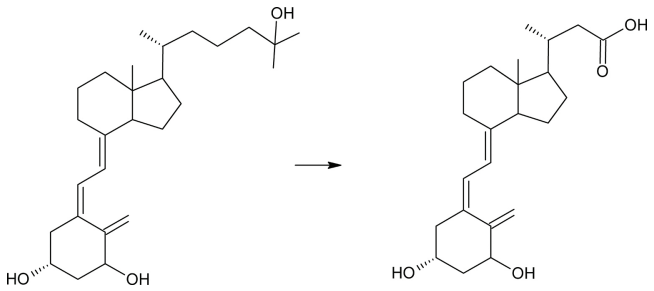

Figure 5. Image illustrating the inactivation of calcitriol, through conversion in calcitroic acid (drawn in ACD/ChemSketch®).

2.3. Molecular actions of vitamin D's metabolites

Calcitriol exerts its effects through a nuclear hormone receptor known as VD receptor. This receptor is a transcription factor that regulates gene expression that mediates the hormone

biologic activity, and in more recent years was found in tissues that are not involved in maintaining calcium homeostasis and bone health. In fact, VDRs have a broad tissue distribution, being present in organs such as heart, stomach, pancreas, brain, skin, gonads, and immune system cells [22].

Thus, despite its well-known regulation of calcium homeostasis and bone mineralization functions, calcitriol is nowadays associated with many extraskeletal effects including antiproliferative, pro-differentiating, anti-inflammatory, and immunomodulatory effects [7]. In fact, recent studies proved that VD receptors are present in activated macrophages and lymphocytes. Binding of these receptors is directly responsible for the activation of antimicrobial genes [23]. Also, this hormone has the ability to suppress prostaglandin actions and enhance pro-inflammatory cytokines production, displaying a role in ceasing inflammatory process [8].

VD is also associated with the regulation of the proliferation of several cells, as cardiac muscle cells [22].

2.3.1. Vitamin D in calcium homeostasis and bone mineralization

It is well established that vitamin D stimulates intestinal absorption of calcium by activating the signaling pathways for calcium transport across the plasma membrane. VD also stimulates calcium mobilization from bone playing an important role in initiating bone remodeling and repairing.

Recent studies proved that all skeleton cells (chondrocytes, osteoblasts, and osteoclasts) contain the receptors for both VD receptor and the enzyme CYP27B1 required for calcitriol synthesis. Therefore, it was proved that VD plays a major role in the activation of osteoblasts to the osteoclast cells to resorb bone. Also, activated osteoclasts induce the reverse transport of calcium from the bone to plasma.

VD active metabolite is also responsible for altering the expression of several skeletally derived factors as growth hormone that can exert effects on bone homeostasis [3, 24].

2.3.2. Vitamin D and its antineoplastic activity

Several studies support that vitamin D may play a major role in tumor's pathogenesis, progression, and therapy [8–11]. In fact, as Garland and coworkers state, more than 3000 research studies have been published investigating vitamin D and its metabolites antineoplastic activity [18]. The types of cancer where most of the anticancer actions of vitamin D have been studied are the breast, prostate, and colon cancers [10].

As mentioned above, calcitriol exerts its effects through the VDR. This receptor is widely distributed among tumor cells, regulating calcitriol antineoplastic activity [8, 9, 15]. Therefore, several pathways by which vitamin D metabolites may prevent, treat, or stop tumor growth have been described. The most discussed mechanisms are (1) inhibition of tumor cell growth, (2) inhibition of angiogenesis and tumor metastasis, (3) triggering apoptosis, (4) enhancing of "traditional" anticancer agents therapeutic action, and (5) anti-inflammatory effects [8–11].

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1. VDR activation by calcitriol can inhibit tumor cell proliferation by inducing cell cycle arrest in the G_1/G_0 phase [8, 10, 11]. The cell cycle is divided in five different phases. The first stage is called the G_0 phase, a resting phase where the cell has left the cycle and has stopped dividing. Then, the cell enters in the G_1 phase, a checkpoint to ensure that all cellular mechanisms are ready for DNA synthesis that occurs in the next stage, the S phase. Another checkpoint follows the S phase, the G_2 gap. Finally, cell division—mitosis—occurs in the M phase [25]. VDR activation by calcitriol can also inhibit tumor cell proliferation through inducing malignant cells differentiation in a variety of cell lines [8, 9, 11].

2. Calcitriol also inhibits angiogenesis by reducing the proliferation of vascular endothelial cells [8, 11] and regulating the expression of key molecules, such as serine proteinases, metalloproteinases, extracellular matrix proteins, and integrins [8, 9]. Another calcitriol antineoplastic activity is related to reducing the invasive and metastatic potential of tumor cells [8–10]. Inhibition of tumor metastasis is due to increased expression of E-cadherin, a tumor suppressor associated with the metastatic potential of cells, and inhibition of angiogenesis itself [9].

3. Apoptosis triggering of tumor cells occurs through activation of the intrinsic pathway of apoptosis by increasing the expression of proapoptotic proteins and decreasing the expression of antiapoptotic proteins, or by directly activating effectors caspases [10, 11]. Apoptosis may also be induced by the inhibition of telomerase enzyme [11]. This programmed cell death is characterized by causing the disruption of mitochondrial function, cytochrome release, and production of reactive oxygen species [10, 11].

4. Vitamin D can also potentiate the antitumor actions of a number of more "traditional" anticancer agents [8, 11].

5. Inflammatory mediators such as cytokines, chemokines, prostaglandins, and reactive oxygen and nitrogen species enhance tumorigenesis through the activation of multiple signaling pathways in tumor tissue. Hence, anti-inflammatory effect of calcitriol mentioned at the section above can also be considered as an antineoplastic activity [8, 10].

However, calcitriol exhibits antitumoral activity only at supraphysiological doses (10^{-9} to 10^{-6} M *in vitro* and $>10^{-9}$ M *in vivo*) associated with a high risk of hypercalcemia [9, 13].

Not only calcitriol plays a major role in tumor's pathogenesis, progression, and therapy but also the enzymes involved in its metabolism have urged a serious interest in several research projects. Tumor cells, likewise other vitamin D target tissues, exhibit enzyme 24-hydroxylase responsible for calcitriol inactivation. Therefore, vitamin D activity will be reduced. Some studies address this problem through a combination therapy with 24-hydroxylase inhibitors alongside with calcitriol administration. However, this co-addition may also results in an increase in the risk of hypercalcemia effects. For these reasons, several authors argue that structural analogs of calcitriol that resist 24-hydroxylation may be a more useful cancer therapy [8].

It is documented that the enzyme 1-α-hydroxylase, which converts calcidiol into calcitriol, is also present in cancer tissues such as breast and prostate cancer. Therefore, calcidiol also could

be administrated as a therapeutic agent, increasing local drug concentration without systemic side effects associated to calcitriol high levels [8, 13].

2.4. Vitamin D deficiency/insufficiency and health risks

Vitamin D deficiency/insufficiency is a worldwide well-recognized problem associated with an increased risk for several acute and chronic diseases [2]. VD deficiency is a resulting consequence of the modern lifestyle with the prevalence of obesity, increased information, and consciousness on harmful effects of UV radiation leading to an increased sun protection and sun avoidance [23]. Due to the very limited dietary sources of VD and insufficient exposure to sunlight in northern regions, between 30 and 60% of the European and North American population suffer from VD deficiency [12].

Thus, several adverse outcomes are nowadays firmly associated with the VD insufficiency problems. Concerning the musculoskeletal system, low bloodstream levels of VD are related to low bone mineral density leading to rachitic with increased fracture risk. This bone illness is associated more directly with the circulating VD levels than to the dietary calcium intake [23].

VD insufficiency is also a risk factor for the development of various cardiovascular diseases. As VD regulates the proliferation of cardiac muscle cells, its deficiency is associated with the increased risk for coronary artery disease, heart failure, and peripheral artery disease. Studies also showed that VD deficiency leads to the development of hypertension, ventricular hypertrophy, and coronary artery calcification [22].

VD deficiency can also be related to the appearance of microbial diseases since VD plays a major role in promoting antiviral activity [23].

As mentioned before, VD plays a major role in tumor's pathogenesis; therefore, its deficiency increases the risk of developing several types of cancer [26]. In fact, studies show the relationship between VD deficiency and at least 15 types of cancer, including breast, colon, rectal, gastric, and ovarian cancer. VD insufficiency leads to an impairment of antimitogenic, proapoptotic and prodifferentiating signaling pathways that have been implicated in the pathogenesis of these types of cancer [21].

2.5. Nanoparticles for the encapsulation of vitamin D

The recognition that vitamin D deficiency as a health risk leads to the development of new functional foods and therapies using nanotechnologies for VD incorporation into foods and pharmaceutical formulations without reducing its bioavailability or activity.

Nanomedicine has dictated trends in the last decades, and its influence is notorious in several fields, since nanomaterials exhibit unique physicochemical properties due to their small size and larger surface area. Nanoparticles (NPs) are colloidal carriers with dimensions on the nanoscale (10^{-9} m) with unique physicochemical properties as small size, larger surface area, stability, varied composition, biocompatibility, and biodegradability [27, 28]. Encapsulating molecules in a nanocarrier allows to increase their bioavailability and bioaccumulation in the target site, and decreases their toxicity. The fulfillment of these main goals allows maximizing

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therapeutic effects and minimizing side effects [27, 28]. Nanoencapsulation of several compounds can be achieved using a wide variety of different nanocarriers. At the moment, the most studied NPs are liposomes, polymeric NPs, dendrimers, lipidic NPs, micelles, carbon, and silica nanotubes.

Among polymeric NPs, poly(D,L-lactide-co-glycolide) (PLGA) is probably the most popular ones. PLGA has become one of the most attractive candidates for a range of applications due to being biocompatible, biodegradable, and Food and Drug Administration (FDA)-approved, and having adjustable biodegradation rate and tunable mechanical properties [29]. PLGA allows the controlled and sustained release of VD for several days, increasing VD's bioavailability and enhancing its therapeutic effect [30]. However, its use faces a few limitations due to their poor loading capacity, allowing the delivery of only about 10% (w/w) of VD, as already described [30]. The characteristic initial burst release can be another major pitfall since a large amount of VD is lost before reaching the target tissue [30]. Also, the many required steps for NP production such as centrifugation and dialysis are expensive and difficult to scale up. PLGA NPs also exhibit a size-dependent cytotoxicity. Small PLGA NPs may trigger the generation of reactive oxygen species, mitochondrial depolarization, and inflammatory cytokines release. Another drawback of these polymeric NPs is the challenge of hydrophilic molecules entrapment, since those rapidly partition into the aqueous phase during NPs preparation. For that is necessary to use appropriate preparation methods as the double emulsion technique [29].

On the other hand, liposomes offer an advantage in the encapsulation of hydrophilic molecules, since they have the ability to carry hydrophilic and hydrophobic drugs within the aqueous vesicles and lipid bilayer membranes, respectively. Liposomes are probably the most popular among the nanosystems studied for nanomedicine applications. Liposomes are small spherical vesicles composed of concentric bilayers of self-assembled phospholipids in aqueous medium and can be classified into different categories by their number of bilayers and size.

Multilamellar vesicles (MLVs) are composed of a structure of concentric phospholipid bilayers separated by water compartments. In unilamellar vesicles (UVs), the liposomes exhibit only a single phospholipid bilayer enclosing the aqueous compartment. These unilamellar vesicles can also be classified into two different types: the small unilamellar vesicles (SUVs) and the large unilamellar vesicles (LUVs). Liposomes can be produced in a broad range of sizes, from 15 to 2000 nm. The most used technique for the preparation of liposomes is Bangham's method. This method consists in the preparation of lipid moisture and evaporating it to form a lipid film. Then, the film is hydrated to form liposomes. The product of hydration is large MLV. Liposomes can be downsized by a variety of techniques, including sonication or extrusion. While usually sonication yields SUV (in the range of 15–50 nm), extruded liposomes are usually LUV [31].

Liposomes are nontoxic and biocompatible causing no harmful effects to the human body, as they are very similar in structure and composition to the cell membrane phospholipids. Such nanocarriers are considered excellent systems for drug-controlled release due to their structural flexibility, size, composition, and fluidity/permeability of the lipid bilayer versatility. Also, their surface is easily functionalized to their polar head groups [32]. However, liposomes

present some disadvantages as well as low solubility, short half-life, and high production costs. Also, the phospholipids can undergo oxidation, and in several cases leakage of encapsulated molecules is verified, especially in low-molecular-weight molecules [33].

Since all systems have advantages and disadvantages, the choice of the most suitable nanosystems must take into account the molecule to be encapsulated and further application.

Several studies using nanocarriers for VD delivery both for therapeutic and for food fortification usage have been reported. Some of these studies are summarized in **Table 1** and **Table 2** and discussed below. These nanosystems will allow maintaining the physical and chemical stability of VD, protecting the molecule from extreme temperatures, light, and oxygen that food and pharmaceutical products may be exposed to.

Nanocarrier	Indication	Development phase			Ref.
		Physicochemical studies	Release studies	Cellular studies	
Liposomes	Cheese fortification	EE, TEM, VD recovery rate	n/a	n/a	[33]
Alginate NPs	Oral administration	FTIR, NMR, DLS, TEM, EE	SGF	n/a	[34]
Chitosan-zein NPs	Food fortification	SEM, FTIR, DSC, DLS, ELS, EE, stability	SGF	n/a	[35]
O/W emulsion	Cheese fortification	Stability, VD recovery rate	n/a	n/a	[36]
Micelles	Food and beverage fortification	Stability, DLS	n/a	n/a	[37]
Chitosan/soy protein NPs	Food fortification	DLS, ELS, SEM, EE, FTIR	SGF	n/a	[38]
Protein NPs	Food and beverage fortification	DLS, stability	n/a	n/a	[39]
Chitosan micelles	Food fortification	FTIR, NMR, XRD, DSC, TEM, AFM, ELS, DLS, stability, EE	SGF and PBS	Fibroblast mouse cell line (L929); MTT assay	[40]
Nanoemulsion	Food and beverage fortification	DLS, stability	n/a	n/a	[41]

Note that n/a stands for not applicable, AFM for atomic force microscopy, DLS for dynamic light scattering (used for size determination), DSC for differential scanning calorimetry, EE for NPs encapsulation efficiency, ELS for electrophoretic light scattering (used for zeta potential determination), FTIR for Fourier transform infrared spectroscopy, NMR for nuclear magnetic resonance, PBS for phosphate buffered saline, SEM for scanning electron microscope, SGF for simulated gastrointestinal fluid, TEM for transmission electron microscope and XRD for X-ray diffraction. SRB is a cellular proliferation assay (colorimetric) and MTT is cellular viability assay (colorimetric).

Table 1. Currently developed nanosystems for the entrapment of vitamin D for food fortification.

Nanocarrier	Indication	Development phase			Animal studies	Ref.
		Physicochemical studies	Release studies	Cellular studies		
PLA NPs	Cancer treatment	DLS, EE, stability	PBS	Human breast cancer cells (MCF-7); MTT assay; cellular uptake (Fluorescence microscopy)	n/a	[13]
PLGA NPs	Cancer treatment	DLS, ELS, TEM, stability	PBS	Human pancreatic cell lines (S2-013 and hTERT-HPNE); lung cancer cell line (A549); SRB assay, cellular uptake and morphology (confocal microscopy); flow cytometry (cell cycle analysis)	n/a	[45]
HAp-PLGA NPs	Osteogenesis and bone tissue differentiation	XRD, FTIR, DLS, ELS	n/a	Mouse calvarial preosteoblastic cell line (MC3T3-E1), confocal microscopy	Rats with osteoporosis and induced bone defects, pathohistological analysis of bone tissue after sample injection	[44]
Quantum dots	Cancer diagnosis and treatment	FTIR, AFM	n/a	Mouse myoblast cell line (C2C12), confocal microscopy, luciferase activity assay (gene expression)	n/a	[43]

Table 2. Currently developed nanosystems for the entrapment of vitamin D for therapy.

As VD is a lipophilic component, it is necessary to be incorporated into aqueous media to become suitable for food and beverage products; also, fat removal during food products processing results in the removal of fat-soluble micronutrients, as VD [2]. Thus, the fortification of food and beverages with VD can be envisaged by its encapsulation in nanoparticles. It is important that the developed delivery nanosystem does not alter the physical, chemical, or sensory properties of the food or beverage product that it is incorporated.

Banville and coworkers used liposomes for the supplementation of Cheddar cheese with vitamin D. The attained nanosystems allowed to maintain the stability of VD for up to 5 months. The group used multilamellar liposomes to achieve high encapsulation efficiency values (approximately 80%). VD-loaded liposomes were added to milk before cheese produc-

tion, and the authors verified that VD was recovered in cheese with high recovery rates (60%) when compared to control conditions. The entrapment of VD in the liposomes allowed to obtain Cheddar cheese enriched with high levels of VD not altering the chemical composition of the fortified cheese [34].

Li and colleagues developed alginate derivate NPs, as a carrier for oral administration of VD, to enhance its water solubility improving its bioavailability. Alginate was modified with oleoyl chloride yielding oleoyl alginate ester conjugate for the NP preparation. The group verified that by increasing the concentration of used oleoyl chloride, the size of the obtained NPs decreased from 500 to 300 nm, approximately. The developed system exhibited high encapsulation efficiency values (approximately 70%) and maintained their structural and chemical properties in simulated gastrointestinal fluids. The NPs exhibited a controlled and sustained release of VD in the simulated human body fluids. The attained results proved that the developed nanosystem is a suitable oral carrier for the delivery of VD [35].

Luo and coworkers encapsulated VD into chitosan-zein NPs for food fortification to increase its stability and health-promoting properties during processing and storage. The group prepared zein nanoparticles with a chitosan surface's coating. Zein has been extensively studied for its ability to form biodegradable, biocompatible, and nontoxic NPs. Coating with chitosan significantly enhanced the NPs encapsulation efficiency from 50 to 90% approximately. Calcium was used as a cross-linker, and its influence in the NPs mean size was assessed. The group verified that the mean size varied from 80 to 200 nm, increasing the size with the increase of calcium concentration. The prepared NPs showed a controlled release of VD in both PBS medium and simulated gastrointestinal fluid proving to be a suitable system for the oral delivery of VD [36].

Tippetts and colleagues incorporated VD in oil-in-water emulsion, using milk protein emulsifiers to fortify milk for cheese production. The authors verified that the retention of vitamin D in cheese was enhanced when using the nanoemulsion comparatively with free vitamin. The obtained results proved that this nanosystem is suitable for milk fortification with VD for cheese production [37].

Ziani and colleagues developed surfactant-based colloidal delivery systems for VD, and other lipophilic active agents, encapsulation for food and beverage products fortification strategies. The group prepared oil-in-water emulsions and studied the influence of the surfactant type on the incorporation of VD into the surfactant micelles. The surfactants were Tween 20, 60, and 80, respectively. The study provided valuable knowledge for the rational design of delivery systems for food fortification [38].

Teng and colleagues successfully incorporated VD in carboxymethyl chitosan and soy protein complex nanoparticles to improve water solubility, absorption, and protection for food products fortification. The effect of pH and chitosan/soy protein mass ratio on the formation of nanoparticles was studied. The attained nanovehicles exhibited sizes around 200 nm and encapsulation efficiency values around 90%. The prepared nanoparticles showed a successful release of VD in simulated gastric fluid and under simulated intestinal condition proved to be a suitable VD nanocarrier for food industry application [39].

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Abbasi and coworkers developed protein isolate nanoparticles for the encapsulation of VD. The group concluded that the incorporation of VD in this nanocarrier allows delaying its degradation during storage time, and therefore they can be considered as enriching agent in beverages, fruit drinks, or low-fat food [40].

Li and coworkers encapsulated VD in chitosan-derived micelles to improve the solubility, efficacy, and stability of VD for functional food products. The attained nanosystem showed mean diameters around 200 mm and encapsulation efficiency values of approximately 50%. The prepared micelles also exhibited a biphasic release profile, with an initial rapid release, followed by a sustained release. The cytotoxicity of the nanocarrier was assessed using fibroblast mouse cells, and the results showed that the chitosan micelles had low cytotoxicity against the studied cell lines, proving to be biocompatible [41].

Guttoff and colleagues developed stable delivery systems for VD with high oral bioavailability based on nanoemulsions. The main goal of this work was to incorporate VD into aqueous-based food products, such as waters or juices. The authors assessed the influence of several experimental conditions on the nanoemulsion-obtained characteristics, such as the surfactant-to-oil ratio and surfactant type. The group prepared nanoemulsions with droplet diameters under 200 nm, stable for at least 1 month at storage conditions (room temperature). The results suggested that the developed nanosystem will be suitable for food and beverage fortification with VD [42].

Despite calcitriol's multiple medicinal benefits, its low bioavailability and high toxicity continue to be highlighted as major challenges in developing formulations for clinical use. In fact, two pharmaceutical formulations for Rocaltrol® (registered trademark of Roche Pharmaceuticals) are available with different administration pathways, oral and intravenous, for the treatment of refractory malignancies. However, they are inappropriate for cancer treatment due to several technical issues, as the difficulty to maintain active systemic levels [13, 43]. Also, several studies indicate that more than 75% of vitamin D intake is catabolized and excreted before being converted to its active form or before its storage. After being absorbed by the intestinal mucosa, vitamin D suffers first-pass effect being conducted by the portal vein to the liver where it is metabolized by hepatic 24-hydroxylase enzyme. This enzyme inactivates calcitriol by hydroxylation, yielding calcitroic acid as an inactive metabolic product. Therefore, vitamin D concentration is greatly reduced before it reaches the systemic circulation, and consequently before it reaches target tissues [14].

Also, some studies, intending to use VD as a therapeutic agent, aiming to increase its bioavailability avoiding first-pass effect, and decreasing its toxicity by ensuring specific action on target cells, have been reported. As calcitriol exhibits antitumoral activity only in supraphysiological concentrations as mentioned above [9, 13], its encapsulation on NPs could address the toxicity issue. One of the main advantages in using NPs to cancer therapy is the enhanced permeability and retention (EPR) effect verified in tumor tissues. The NPs take advantage of the increased permeability of blood vessels in tumor tissues, whereas lymphatic drainage is decreased which increases the concentration of loaded nanoparticles in the tumor tissue. As the EPR effect does not occur in healthy tissues, it is thus possible to target tumor cells, reducing VD's toxicity in healthy tissue [44].

Almouazen and partners encapsulated calcidiol in poly-lactic acid (PLA) nanoparticles to ensure specific action on malignant cells avoiding side effects as hypercalcemia. The authors developed nanocapsules with about 200 nm of mean diameter. Cellular studies showed a significant growth inhibition when calcidiol was entrapped in the PLA nanocapsules, when compared to free calcidiol, proving that the nanocarrier enhanced the intracellular delivery of vitamin D on breast cancer cells. The attained results showed that PLA nanocapsules are a suitable choice for the controlled delivery of calcidiol [13].

Bonor and coworkers developed calcitriol-conjugated quantum dots to study the distribution of calcitriol in mouse cancer cells. The designed tool is suitable for imaging drug-tumor interactions and to deliver drugs to tumors and metastasized sites [45].

Ignjatović and colleagues prepared hydroxyapatite (Hap) and PLGA-based nanoparticles for the local delivery of VD to enhance osteogenesis and bone tissue differentiation. The attained NPs exhibited mean diameters of 100 nm and a biphasic release profile. *In vitro* biocompatibility studies were conducted using osteoblastic cells. In animal studies, the authors verified that osteogenesis and bone structure differentiation were enhanced when VD was delivered by the developed system [46].

Ramalho and colleagues developed PLGA nanoparticles for the delivery of calcitriol for an antitumor therapy application. Initially, the authors used cholecalciferol as drug model for calcitriol to assess the influence of several experimental conditions, such as sonication time and VD/polymer ratio, on the NPs physicochemical properties. After achieving the optimized experimental conditions, the group synthesized calcitriol-loaded PLGA NPs with spherical form and mean diameters smaller than 200 nm as shown in **Figure 6**, and stable for several weeks at storage conditions (4°C). The attained nanosystems exhibited encapsulation efficiency values of approximately 60%. The prepared PLGA NPs exhibited a biphasic release profile, with an initial burst release in the first 24 h, followed by a slower and controlled release for 7 days. Human cancer cell lines were used to evaluate the toxicity of VD-loaded PLGA NPs. The obtained nanoparticles formulation was successfully internalized by the target cells and enhanced the vitamin's antitumor effect, showing a clear efficacy in the therapeutic effects as cell cycle arrest and major changes in cell's morphology [30].

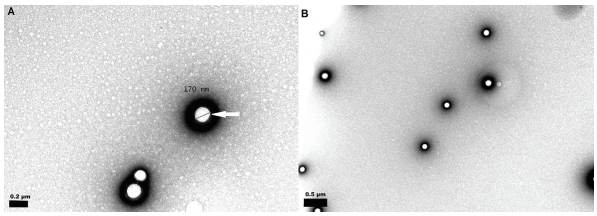

Figure 6. TEM images: (a) unloaded PLGA nanospheres; scale bar: 200 nm; (b) VD-loaded PLGA nanospheres; scale bar: 500 nm [30].

These developed systems reported in the literature allowed maintaining active doses of VD for long periods of time, due to their controlled and sustained release. These nanosystems also

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showed the ability to reduce and destroy tumor cells, taking advantage of the EPR effect verified in tumor tissues. As the EPR effect does not occur in healthy tissues, it is thus possible to target tumor cells, reducing VD's toxicity in healthy tissue [44]. None of the works here discussed reported the use of functionalized NPs. Modification of the NPs' surface with antibodies or other specific molecules would allow to address a more efficient therapy, enabling a targeted distribution into specific tissues.

3. Conclusion

With the growing awareness of vitamin D health benefits, as well of the harmful risks associated to vitamin D insufficiency, finding new solutions has become urgent within the scientific community. In more recent years, nanotechnology has emerged as a suitable answer to these issues, allowing to take advantage of the beneficial effects of this micronutrient, while overcoming some of the disadvantages associated with its administration. Nanoparticles provide protection from external conditions, and increase the stability and solubility of the molecule. Also, nanoparticles allow decreasing its toxicity associated with the hypercalcemia phenomena, and allowing circumventing the multidrug resistance problem hindering the molecule efflux out of the cells. Only a few nanosystems have been described for different applications, such as food and beverage fortification and as therapeutic agents, as shown in this chapter. It would be essential to conduct more substantial and insightful studies to support the great potential of nanotechnology for the delivery of vitamin D. Also, it would be valuable to optimize the already-described systems to make them more efficient and specific to a specific target tissue.

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