AAPS PharmSciTech (2019) 20: 11
DOI: 10.1208/s12249-018-1231-9

Review Article

Theme: Lipid-Based Drug Delivery Strategies for Oral Drug Delivery
Guest Editor: Sanyog Jain

Recent Advances in Formulation Strategies for Efficient Delivery of Vitamin D

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Received 16 August 2018; accepted 26 October 2018; published online 17 December 2018

Abstract. Deficiency of vitamin D is a global concern affecting a huge number of human populations. This deficiency has a serious impact on human health not only affecting bone mineral density but also becoming the reason for cardiovascular disorders, infectious diseases, autoimmune diseases and cancers. Exposure to sunlight is the major source of vitamin D, but due to the present day-to-day lifestyle of working in a shade arouses the need for exogenous sources of vitamin D. Ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are the two major forms of vitamin D, which are hydrophobic in nature and highly susceptible to environmental conditions, like temperature and light. Therefore, novel drug delivery systems could be explored for efficient delivery of vitamin D. In this review, a brief account of vitamin D is provided followed by a detailed description of recent advances in various delivery systems, including solid lipid nanoparticles, nanoemulsion, self-emulsifying drug delivery systems, polymeric nanoparticles and solid dispersion, for the efficient delivery of vitamin D.

KEY WORDS: nanoformulations; vitamin D; ergocalciferol; cholecalciferol; solid lipid nanoparticles.

INTRODUCTION

Nutritional deficiencies and inadequacies have become a major health issue all across the globe. Deficiency in vitamin D, folate and iron is noted among adolescents, children, pregnant women and elderly (1). Deficiency of vitamin D is one which always remains underdiagnosed due to false perceptions that a few minutes in sunlight and regular diet provide sufficient amount of vitamin D (2). High-altitude areas, socio-religious cultural practices, seasonal changes, sun avoidance and excessive use of sunscreen are some of the limitations responsible for vitamin D deficiency. The scarcity of vitamin D in natural products has led fortification of staple foods as the most prominent and viable strategy to overcome deficiency; unfortunately, this strategy has failed till date, as only a few fortified products are available in the market. Fortification is the only option, which if utilized at a large scale can become successful for providing 2000 IU daily dose requirement and help in reducing the menace of associated diseases (3).

Biological Functions

Vitamin D is an essential micronutrient that enables small intestine to absorb calcium and phosphorus from food sources. Absorbed minerals are required for normal cellular functions in all cells, nerve conduction, muscle contraction and mineralization of the bone; so, deficiency of vitamin D is a major menace causing rickets, osteomalacia, hyperparathyroidism and osteoporosis (4,5). New analogues of vitamin D have been developed and under clinical trial for its efficiency in the treatment of psoriasis. Such activity actually relies on its immuno-modulatory action of inhibiting cellular proliferation and differentiation (6). Type 1 and type 2 diabetes mellitus patients are reported being in jeopardy if they are vitamin D deficient, as chances of cardiovascular mortality, insulinemia and glucose intolerance increase (7–9; Fig. 1). The efficiency of vitamin D analogues as an anticancer agent has also been reported due to its ability in inhibition of proliferation, differentiation and angiogenesis (10–11).

Recommended Dietary Intake

The concentration of 25-hydroxyvitamin D [25(OH)D] in serum indicates vitamin D status, and minimum required concentration is 20 ng/ml (12; Table I). As per the Institute of Medicine (US), a study on Germans (aged 20–70 yrs) showed no sign of vitamin D deficiency in those having blood concentration of 25(OH)D more than 20 ng/ml. However, there is also some literature which observed the evidence of

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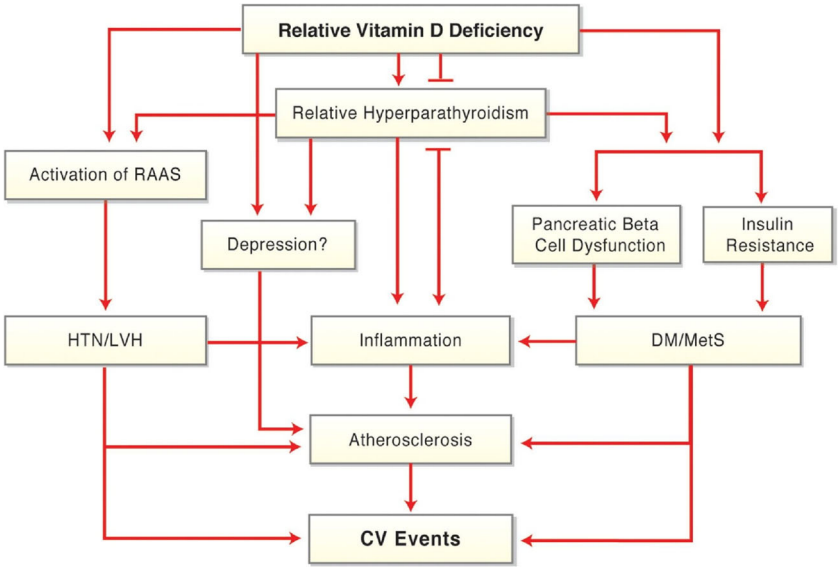

Fig. 1. Cardiovascular events on vitamin D deficiency. CV, cardiovascular; DM, diabetes mellitus; HTN, hypertension; LVH, left ventricular hypertrophy; MetS, metabolic syndrome; RAAS, renin-angiotensin-aldosterone system. Reproduced with permission from Elsevier (7)

increased osteoid in men and women with a concentration of 20–30 ng/ml of 25(OH)D in the blood. As per few reports, 34 and 38 ng/ml of 25(OH)D are required for calcium absorption from the intestine and for performing neuromuscular function; so, 40 ng/ml was the final concentration which was decided to prevent hyperparathyroidism (13). The nutrient guidelines published by US and Canada depicting dietary requirement of vitamin D were presented in Table II (14).

Risk Factors

The sunshine vitamin requires regular exposure to sunlight for natural synthesis, but the recent lifestyle of indoor daytime job culture, homeboundness and wearing long sleeves and head coverings against the fear of tanning prevents its synthesis (Fig. 2). Excessive use of sunscreen has become a problem since their composition is designed to filter UVB rays which are essential for vitamin D production (15,16). People with darker skin tone are more prone to less absorption of UVB light, thus necessitating higher exposure to produce an equal quantity of vitamin D as required by those with fairer skin tone (17). Peoples with impaired fat absorption and bariatric patients are not able to absorb fat-soluble vitamins. In one study, it was justified that people with high body mass index (BMI) value are mostly observed with lower serum 25(OH)D concentration compared to those with low BMI value and so required larger vitamin D intake (18). High antagonistic effect was reported by the administration of phenobarbital and phenytoin on vitamin D-associated effects as both of these drugs induce the rapid metabolism of vitamin D (19,20). The presence of severe condition of hyperparathyroidism and granuloma-forming disorders also serves rapid metabolism of vitamin D (21). Breastfed infants are more susceptible to vitamin D deficiency, and for this, the American Association of Pediatricians (AAP) recommended 400 IU of vitamin D per day to them (22). Ageing also reduces the capacity of vitamin D synthesis due to a decrease in 7-dehydrocholesterol precursor in the skin (23).

Population Under Risk Factor

Vitamin D deficiency is primarily noted among children, young person, pregnant women and elderly (1). In the Indian subcontinent, 70–100% of the general population was observed to be affected by vitamin D deficiency and this rate is common in both rural and urban populations (2). The report published by the International Osteoporosis Foundation declared the prevalence of deficiency in 78% of individuals living in North India while in south India, maximum deficiency was observed in females (70%) rather than males (24). Prevalence of deficiency with rate of 80.9% was observed in women with age over 80 years in the European continent (25). In the American continent, its deficiency is prevalent in 42% of adult, 82% of black individuals and 69% of the Hispanic population (26).

VITAMIN D COMPOUNDS

Food products like mushrooms and fish are two main sources that are composed of vitamin D but only at very minute quantity. Even non-availability of sufficient fortified milk and fortified cereals has made the condition worse (27). So, dependence on supplements has become necessary. Vitamin D supplements are available in the form of ergocalciferol (vitamin D_2), cholecalciferol (vitamin D_3) and vitamin D analogues.

Vitamin D_2 (Ergocalciferol)

Ergocalciferol is produced from plant sources, especially mushroom and plants contaminated with fungi. In plants, ergocalciferol is photosynthesized in the skin of plants by exposure to UV irradiation of ergosterol (28). Vitamin D_2 is a

Table I. Concentration of 25(OH)D in Blood with Associated Health Impact[a]

nmol/l	ng/ml	Health status
< 30	< 12	Vitamin D deficiency leading to rickets in infants and children and osteomalacia in adults
30 to < 50	12 to < 20	Inadequate for bone and overall health
≥ 50	≥ 20	Adequate for bone and overall health
> 125	> 50	Potential adverse effects to such high levels, particularly > 150 nmol/l (> 60 ng/ml)

[a] Obtained from the Fact sheet for Health professionals prepared by the National Institutes of Health, Office of Dietary Supplements (12)

fat-soluble vitamin that appears as odourless white crystals with a very poor solubility (0.05 mg/ml). The stability of the pure form of vitamin D_2 is also one of the biggest issues because it readily gets oxidized and inactivated in a moist atmosphere at a short span of a few days. Ergocalciferol, as such, is inactive and needs two hydroxylation before the appearance of the active form. The complex metabolic pathway of ergocalciferol involves the initial production of ercalcidiol (25-hydroxyergocalciferol) on exposure to an enzyme present in the liver and the final production into ercalcitriol (1,25-dihydroxyergocalciferol) in the kidney. The plasma concentration of 25-hydroxyergocalciferol is considered by clinicians as an amount of vitamin D_2 in the body while ercalcitriol (1,25-dihydroxyergocalciferol) is the active form of vitamin D_2 in the body (29,30). Considering highly complex metabolic pathway and poor solubility, enhancing dissolution is the best option for increasing bioavailability. Despite the effectiveness of ergocalciferol in treating vitamin D deficiency, the product is not considered equivalent to vitamin D_3 due to differences in elevating serum 25-hydroxyvitamin D, low binding of D_2 metabolites to vitamin D-binding protein and, particularly, non-physiological metabolism of vitamin D_2 (31). Differences in effectiveness of both D_2 and D_3 rely on its rate of metabolism. The presence of an extra methyl group on carbon 24 of ergocalciferol reduces its efficiency in conversion to serum 25(OH)D and also its affinity for vitamin D-binding protein and VDR (32–35). There is one report focusing on the production of an additional product $1,24,25(OH)_3D$ by 24-hydroxylation after 25-hydroxylation in the kidney, and it is this product which in reality demarcates ergocalciferol with cholecalciferol. After the formation of $1,24,25(OH)_3D_2$, ergocalciferol got deactivate and becomes irretrievable while $1,24,25(OH)_3D_3$ retains its property of binding to VDR and still needs an extra side-chain oxidation for deactivation (36). In a study by Heaney *et al.*, weekly doses of 50,000 IU (for 12 weeks) lead to higher value of AUC in the case of cholecalciferol than those for ergocalciferol and discontinuing the doses after 12 weeks showed higher rates of degradation of serum $25(OH)D_2$ than $25(OH)D_3$ during a 6-week period (37). Commercial multivitamin preparations may contain either vitamin D_2 or D_3, but there are emerging trends to formulate vitamin D product containing the vitamin in the form of D3 (Table III). The shift towards vegan is the new trend prevailing in the world, and India is the only country where 35% of its people are strict vegetarians who do not consume animal products (42–43). Such data is quite significant as in India being vegetarian is associated with religious beliefs. Considering such diet issues with the increase in vitamin D deficiency, it has become very much essential to develop and improve formulations of

Table II. Dietary Reference Intakes for Vitamin D and Calcium[a]

Life stage	Vitamin D			Calcium		
	EAR (IU/day)	RDA (IU/day)	Upper level intake (IU/day)	EAR (mg/day)	RDA (mg/day)	Upper level intake (mg/day)
Infants 0 to 6 months	200	200	1000	400	400	1000
Infants 6 to 12 months	260	260	1500	400	400	1500
1–3 years old	400	600	2500	500	700	2500
4–8 years old	400	600	3000	800	1000	2500
9–13 years old	400	600	4000	1100	1300	3000
14–18 years old	400	600	4000	1100	1300	3000
19–30 years old	400	600	4000	800	1000	2500
31–50 years old	400	600	4000	800	1000	2500
51–70 years old males	400	600	4000	800	1000	2000
51–70 years old females	400	600	4000	1000	1200	2000
> 70 years old	400	600	4000	1000	1200	2000
14–18 years old, pregnant/lactating	400	600	4000	1100	1300	3000
19–50 years old, pregnant/lactating	400		4000	800	1000	2500

[a] Modified from Ref. (14)

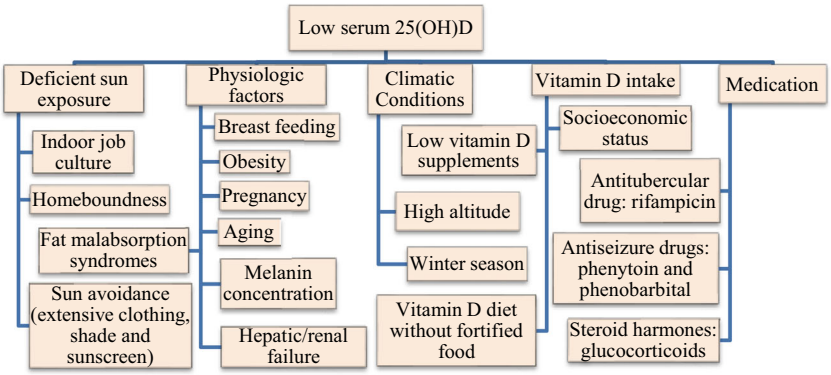

Fig. 2. Risk factors associated with vitamin D deficiency (18)

ergocalciferol, a plant-based vitamin D, to completely eradicate the prevalence of vitamin D deficiency from the society.

Vitamin D_3 (Cholecalciferol)

Vitamin D_3 is an animal-based natural molecule produced as derivative of 7-dihydroxycholesterol on exposure of the skin to UV radiations (44). Fat-soluble nature reduces its solubility (0.013 mg/ml) in aqueous solution. Like vitamin D_2, its stability in moist air is the biggest issue. Vitamin D_3 in circulation bound to its binding proteins and transported to the liver, the site where it gets converted to calcifediol (25-hydroxycholecalciferol). Finally, when calcifediol reaches the kidney, with the help of hydroxylase, it produces an active form known as calcitriol (45). The physicochemical properties like solubility, lipophilicity and dissociation constant of D_3 are almost similar to those of D_2, but the difference is only at metabolism and targeting.

Table III. Study Characteristics of Ergocalciferol and Cholecalciferol

Intervention, dose and frequency	Sex & age (*n*, number of patients)	Follow-up	Results	Reference
a) 1 capsule of 50,000 IU vitamin D_2 b) 5 capsules of 10,000 IU vitamin D_3	F 30 y M 49.5 y ($n = 33$)	12 weeks	12-wk-induced AUC was significantly greater for the vitamin D_3 supplementation group than for the vitamin D_2 group ($P < 0.001$). Vitamin D_3 was calculated as 87% more potent at raising 25(OH)D.	Heaney *et al.* (37)
a) Single oral dose of 300,000 IU vitamin D_3 b) Single intramuscular dose of 300,000 IU vitamin D_3 c) Single oral dose of 300,000 IU vitamin D_2 d) Single intramuscular dose of 300,000 IU vitamin D_2	All F 66–97 y ($n = 32$)	60 days	Vitamin D_3 significantly more potent at raising serum 25(OH)D concentrations than was vitamin D_2 for both oral and intramuscular administration.	Romagnoli *et al.* (38)
a) No supplement (seasonal effect acting as control) b) 1 tablet of 50,000 IU (1.25 mg) of vitamin D_2 c) 10 tablets of 5000 IU (125 μg) of vitamin D_3	All M 20–61 y ($n = 30$)	28 days	28-d AUC was significantly greater for vitamin D_3 supplementation group than vitamin D_2 supplementation groups ($P < 0.002$).	Armas *et al.* (39)
a) Ergocalciferol 1000 IU/d b) Cholecalciferol 1000 IU/d	($n = 70$)	3 months	Vitamin D_3 supplementation was associated with a 31% greater increase in concentrations of serum 25(OH)D than was vitamin D_2 supplementation ($P = 0.01$).	Glendenning *et al.* (40)
a) Study 1: single intramuscular injection of 300,000 IU vitamin D_2 b) Study 2: single 100 ml oral dose of 300,000 IU vitamin D_3	Study 1: 43 F and 7 M Study 2: 15 F and 4 M ($n = 69$)	24 weeks	Greater increases in serum 25(OH)D were achieved with vitamin D_3 intervention	Leventis and Kiely (41)

Vitamin D Analogues

The establishment of knowledge that vitamin D hormones function through a nuclear receptor and the presence of these receptors into the tissues is not linked to calcium and bone creates a new hypothesis of its association with other therapeutic areas and becomes the reason for the development of new vitamin D analogues (46,47). The VDR activation led to classical and non-classical effects, in which the classical effects show that it affects over the endocrine system, especially the gut, parathyroid gland and bones. On the other hand, the non-classical effects, especially the cardiovascular, renal, renin-angiotensin-aldosterone, and adaptive and innate immune systems, are obtained on the local target tissues attributed to paracrine/autocrine vitamin D system (48–52). Development of analogues was focused with the aim of having limited effects on mineral metabolism since a great percent of serum 25(OH)D was utilized in mineral metabolism (53). Some vitamin D analogues are already used in clinical settings and proved preclinically beneficial in reducing side effect and differential non-classical effects. These analogues are obtained by chemical modifications and subsequent screening for specific activity (49). Development of new analogues is distinguished as prodrug and active compounds. Ergocalciferol, cholecalciferol, alfacalcidol, doxercalciferol and calcifediol are prodrugs that require enzymatic activation, while natural hormone, calcitriol and newly developed side-chain-modified products, paracalcitol, maxacalcitrol and oxaccalcitriol, are already developed compounds (Table IV, Fig. 3).

FORMULATION APPROACHES

Advances in combinatorial chemistry, biology and genetics have led to the development of various vitamin D analogues from the parent ergosterol and 7-dehydrocholecalciferol present in the epidermis of plants and animals, respectively. The basic difference in both of them is the presence of a single bond between C22 and C23 and the absence of a methyl group at C24 in cholecalciferol as compared to ergocalciferol. New analogues developed were the result of slight modification in the side chain of parent compounds. The long hydrocarbon chains of these compounds impart high lipophilic nature with lipophilicity above 5.5 and might be the reason for poor aqueous solubility. Dissolution is the first step in the process of absorption, so insoluble compounds, although highly permeable, would not be able to show good absorption. Most of vitamin D analogues are either active or prodrug in nature. In the case of prodrugs, initial metabolism at the liver or kidney is essential for conversion into an active moiety but further first-pass metabolism of an active compound leads to decrease in their serum blood concentration. Considering the issues related to stability, dissolution, absorption and metabolism of vitamin D, the development of novel formulation of vitamin D is the need of the hour **(**Fig. 4**)**.

Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are generally considered as a combination of lipid emulsion and polymeric nanoparticles. The solid lipid core matrix of SLNs can entrap lipophilic molecules, like vitamin D_3, and aids in its solubilization within the surfactant-stabilized aqueous solution. SLNs can also aid in the controlled release of a drug, drug targeting and increased drug bioavailability (55). San Martin-Gonzalez and Patel (2012) have formulated polysorbate 20-stabilized tripalmitin solid lipid nanoparticles of ergocalciferol by hot homogenization technique. The increase in concentration of ergocalciferol from 0 to 20% within the lipid phase of SLN dispersion increases clarity. The stability of ergocalciferol was related to its loading within the lipid crystal, thus, protecting it from oxygen and light. These vitamin-loaded SLNs are beneficial for the formation of clear fortified solutions (56). In 2017, Kim *et al.* have focused on the bioavailability enhancement of cholecalciferol by formulating nanostructured lipid carriers using high-pressure homogenization technique (57). The solid lipid sustained-release nanoparticles

Table IV. Vitamin D Analogues and Their Physicochemical Properties[a]

Chemical	Half-life (h)	pKa	Aq. sol. (mg/ml)	Log P	Trade name	Disease indication
Alfacalcidol (1α-hydroxyvitamin D_3)	3	14.39	0.00163	6.68	Alpha D_3, One Alpha, Eins Alpha, Etalpha, Alfarol	Osteoporosis, renal osteodystrophy, secondary hyperparathyroidism, rickets
Calcidiol (25-hydroxyvitamin D3)	288	18.38	0.0022	6.71	Hideroferol, Didrogyl, Dedrogyl, Calderol	Renal osteodystrophy, osteoporosis and rickets
Paricalcitol (1,25-(OH)2 19-nor-dihydroxy-vitamin D2	4–6	14.81	0.0068	5.27	Zemplar	Secondary hyperparathyroidism
Cacitriol (1α, 25-dihydroxy-vitamin D3)	5–8	14.39	0.0067	5.51	Cacijex, Rocaltrol	Renal osteodystrophy, osteoporosis
Doxercalciferol (1α-hydroxy-vitamin D2)	32–37	14.39	0.00168	5.75	Hectorol	Secondary hyperparathyroidism
Oxacalcitriol (22-oxa-1, 25-dihydroxy vitamin D_3	_	_	Insoluble	_	Oxarol injection	Secondary hyperparathyroidism
Falecalcitriol (1,25-$(OH)_2$–26, 27-F_6-vitamin D_3)	_	_	Insoluble	_	Fulsatn, Hornel	Secondary hyperparathyroidism

[a] PubChem compound database (54)

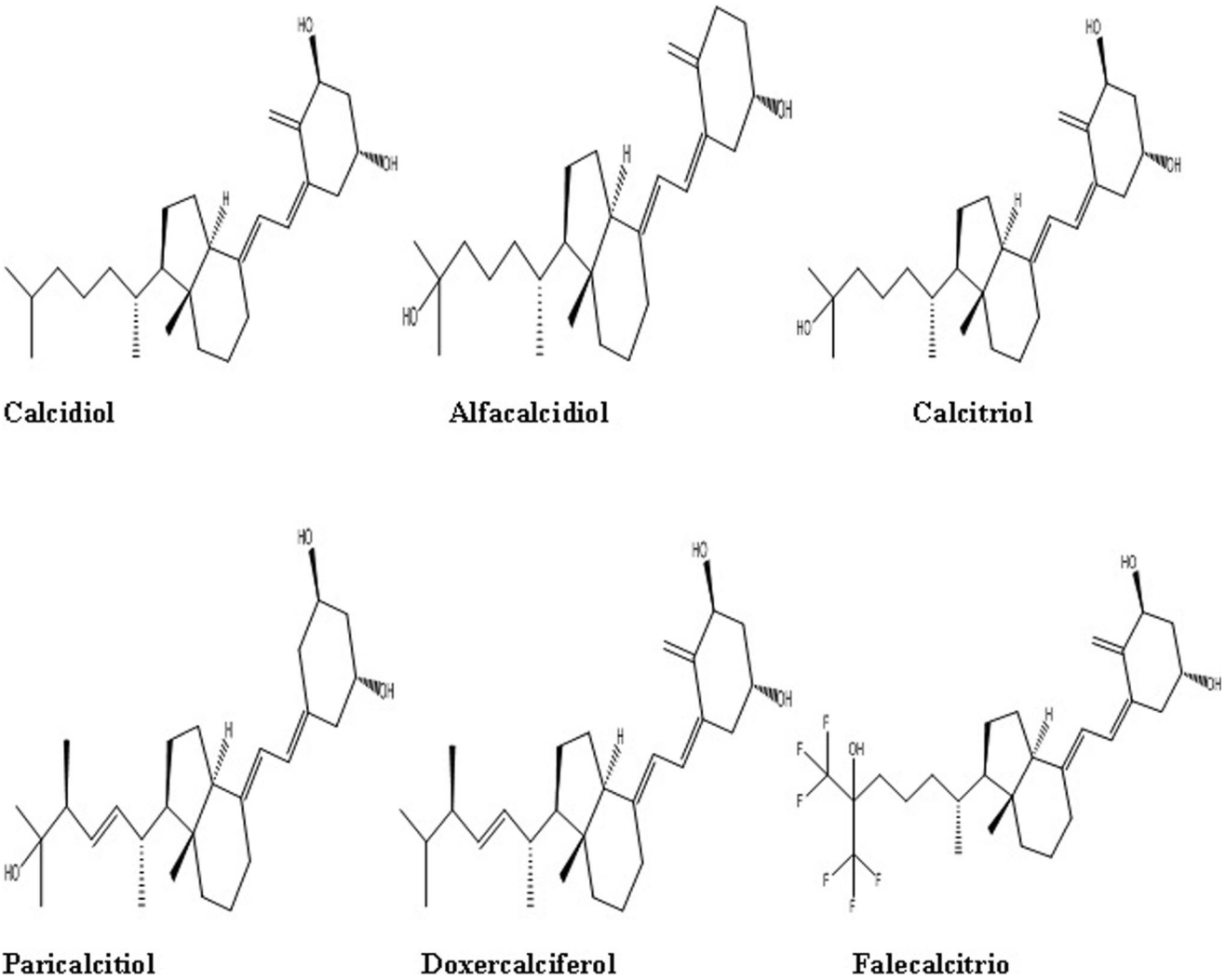

Fig. 3. Structure of vitamin D analogues

of fat-soluble vitamins, especially vitamin D_3 or vitamin D_2, were prepared using microemulsion technique. In this work, a solid lipid core containing entrapped retinoic acid/vitamin D_3 was initially prepared and these lipid cores were then suspended into the solution of surfactant and co-surfactant. From the various formulations, the optimized one was composed of emulsifier, such as polysorbate 80 (45.45%) and soy lecithin (0.58%). Lipid composition (7.27%) was melted at 82–85°C, and the microemulsion so formed was cooled down to form crystals of SLN. The characterization of particles for sustained-release profile confirmed the 100% release of the drug after 7 days (55). The efficiency of solid lipid nanoparticles for the delivery of vitamin D3 was further confirmed by Demirbilek *et al.*, where beeswax-stearic acid blend formulation was used. The particles with size range of 30–60 nm were obtained whose degradation rate, encapsulation efficiency and release rate were increased with increase in concentration of beeswax. The vitamin D3-loaded SLNPs were observed to be immunocompatible and non-cytotoxic to keratinocytes (HaCaT), endothelial (HUVEC) and fibroblast (L929) cell lines (58). The simultaneous administration of vitamin D_3 with all-*trans* retinoic acid (ATRA) entrapped in SLNs was assumed to be beneficial for tuberculosis control with improved and prolonged bioavailability. Vitamin D_3- and ATRA-loaded SLNs were formulated separately but administered simultaneously. Finally, validated ultra-performance liquid chromatography (UPLC) was pre-developed and used for estimation of the drug in the plasma of rat. The pharmacokinetic profile was improved in terms of AUC by 5.4 and 29.4 times for ATRA and vitamin D_3, respectively, in comparison to the plane drug (59). The circulatory movement of lipid-coated cholecalciferol in the blood with its absorption phenomenon was depicted in Fig. 5.

Nanoemulsion

Recently, the attention has been focused on nanoemulsions, submicron emulsions, microemulsion, fine-disperse emulsions and mini-emulsions for improved drug delivery. Based on clarity parameters, nanoemulsions are termed as transparent, translucent and milky. Small size is the only factor which imparts long-term stability (61). McClements *et al.* have developed a nanoemulsion system using spontaneous emulsification method for the oral bioavailability enhancement of vitamin D. The spontaneous emulsification method mainly depends on the formation of tiny oil droplets during the titration of oil/

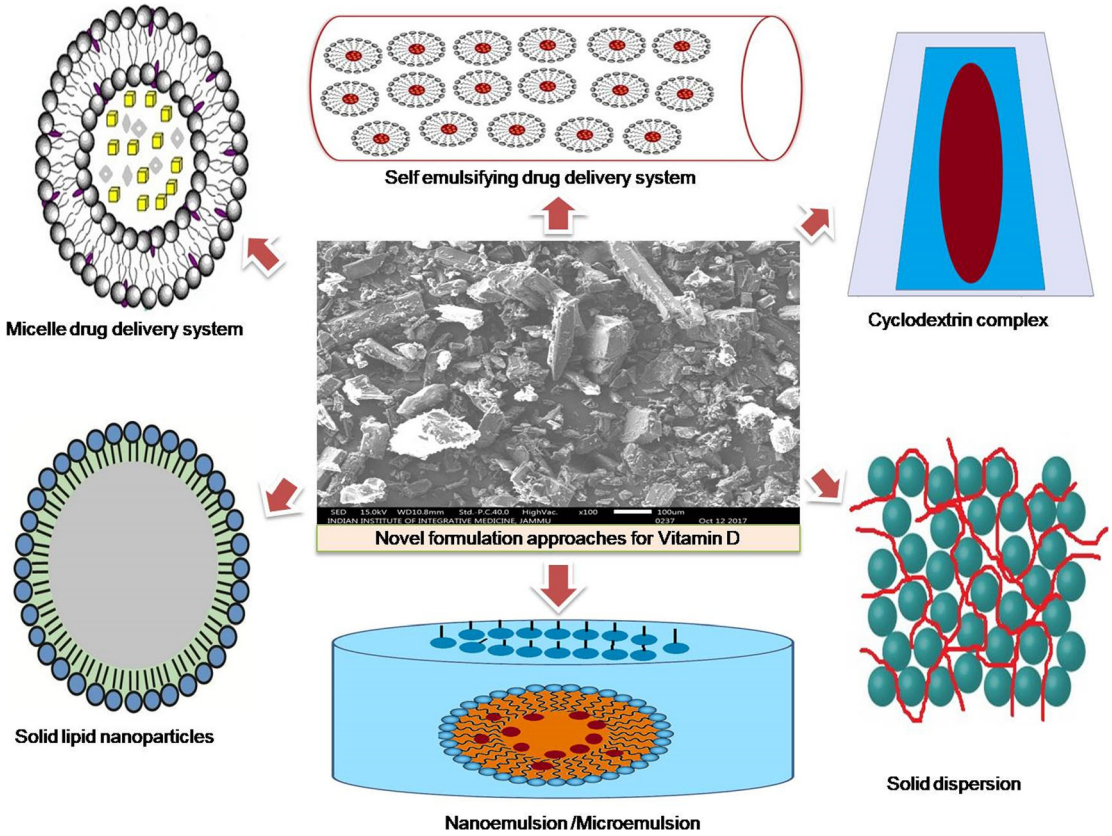

Fig. 4. Formulation approaches for vitamin D

surfactant into an aqueous solution. The smallest particle size droplets were obtained in the nanoemulsions using Tween 80 as a surfactant as compared to Tween 20, 40, 60 and 85, at a stirring speed of 800 rpm. The nanoemulsions were also considerably stable to the growth of droplet at ambient temperature as indicated by less than 10% increase in diameter after one month storage but highly unstable to heating above 80°C (62). The development of nanoemulsions with utmost stability preventing precipitation requires the amphiphilic nature of the polymer which can bind hydrophobic vitamin D with its hydrocarbon chain and get dispersed into the aqueous solution through hydrophilic groups. In one study, the investigators have used chitosan for developing nanoformulation of vitamin D. The selection of modified chitosan irrespective of other hydrophobic polymers was justified on the basis of mucoadhesive properties and presence of positive charges on chitosan. Such feature was important as the efficacy of nanoformulation depends upon the ability to associate with biological tissues (63).

In another investigation, vitamin D formulation was prepared to consist of an inert cavity, an inner layer of cholecalciferol, emulsifier and antioxidant and an exterior layer. The whole system was multi-particulate, where the inner layer was applied directly to an inert core and the exterior protective layer was applied directly to the interior layer. The inner core was a substrate-holding drug layer which prevents its direct contact with an external environment. The inert core might be a granule, a pellet, a bead or a mixture of components, including sugars, starches, polysaccharides or, most preferably, microcrystalline cellulose. The drug was applied in the inner layer in the form of an emulsion which is also composed of an antioxidant. The oil-in-water emulsion was formed where oil was replaced by the organic solvent of medium-chain triglycerides and cholecalciferol to solvent ratio was maintained at 1:30. The addition of emulsifier and its quantity was considered to be essential as chosen to provide a dense layer over an inert core. The concentration of the emulsifier was preferred to be 20% *w*/*w* of the delivery system. Addition of antioxidant in the inner layer was considered to be essential to avoid oxidation of active substances and an organic solvent. The concentration of the antioxidant was in the range 0.4–0.9% *w*/*w*, and the overall fixed ratio of cholecalciferol to antioxidant was 1:4. Some film-forming agent capable of producing a stable solution/dispersion/emulsion and robust layer was also added into the inner layer at 10–20% *w*/*w* concentration. The outer protective layer acts as a coating which prevents the penetration of oxygen, moisture and light inside the pharmaceutical delivery system. HPMC and PVA were some of the polymers which were used at 20% *w*/*w* as an outer layer. The main objective of developing such formulation was to maintain stability as most of the marketed formulations in the form of SEDDS, solid dispersion or nanoemulsion do not show any promising result towards avoiding penetration of moisture, oxygen and light. The confirmation obtained through this invention was that the loss of active substances was not more than 4% under standard accelerated conditions (40°C & 75% RH for 3 months) or intermediate test conditions (30°C & 65% RH for 12 months) (64).

In another interesting investigation, Reboul *et al.* have estimated the effect of dietary fatty acids and their mixtures

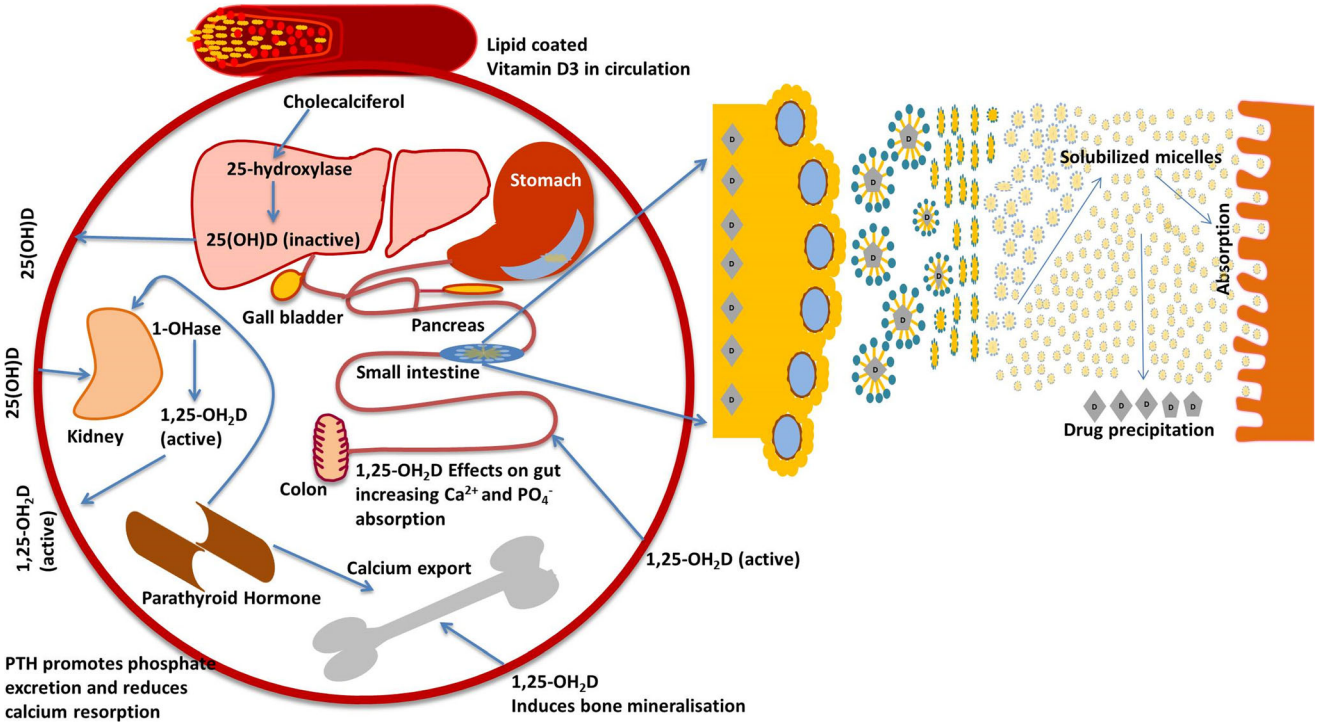

Fig. 5. The oral administration of lipid-based formulations interacts with gastric lipase which initiates its digestion. Simultaneously, propulsion, grinding and retropulsion in the stomach facilitate crude emulsion formation while in the small intestine pancreatic lipase with its cofactor digests the lipids. The products of lipolysis usually in the form of mixed micelles transports into the epithelial cells where chylomicrons and very low-density lipoprotein (VLDL) further enhances its absorption while some of the drug molecules usually precipitated out after lipid digestion (60). Vitamin D_3 in circulation bound to its binding proteins and transported to the liver, the site where it gets converted to calcifediol (25-hydroxycholecalciferol). Finally, when calcifediol reaches the kidney, with the help of hydroxylase, it produces an active form known as calcitriol. Calcitriol in the blood circulation affects small intestine for increased absorption of calcium and phosphate (4)

on the micellar properties of nanoemulsions and absorption of cholecalciferol. It was observed that cholecalciferol being highly lipophilic does not show simple passive diffusion but involved membrane transporters, including scavenger receptor for absorption. The absorption of cholecalciferol in Caco-2 cells was decreased with long-chain fatty acid, while no change in the uptake was observed if mixtures of fatty acid were used. Finally, it was concluded that long-chain FAs, mainly poly-unsaturated free fatty acids (PUFAs), were less effective compared to other FAs, particularly mono-unsaturated free fatty acids (MUFAs), in promoting cholecalciferol absorption but if a mixture of PUFA with other Fas was used, the chances of cholecalciferol absorption would be increased (65). The extent of drug release from nanoemulsion drug delivery system and its absorption from biological membrane depends upon digestion of lipid in the GIT and final micelle formation. The amount of drug dissolved in the micelles is usually assessed as bioaccessibility, which is considered as the amount absorbed from biological membrane. In 2015, Mc Clements *et al.* have investigated the bioaccessibility of cholecalciferol (Fig. 6). In this study, both long-chain triglycerides (LCT), as well as medium-chain triglycerides (MCT), were used for nanoemulsion formulation. The amount of drug in the micelle phase was determined to be more in nanoemulsion composed of MCT as compared to LCT. It was concluded that the chain length of the carrier oil is an important factor for micelle formation rather than free fatty acid release (66).

Self-Emulsifying Drug Delivery System

The self-emulsifying drug delivery system (SEDDS) consists of a mixture of active ingredient, oil, surfactant and co-surfactant

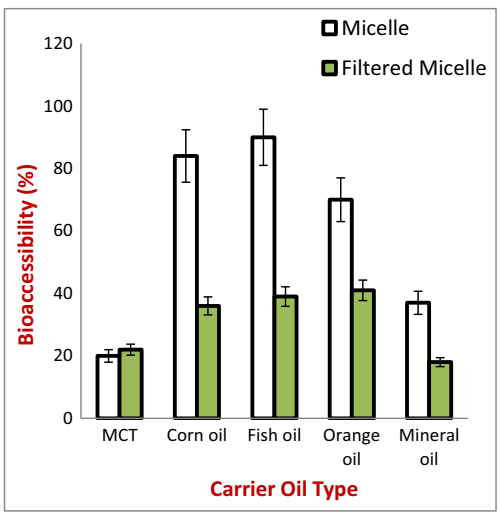

Fig. 6. Influence of carrier oil type on the bioaccessibility of vitamin D3 initially encapsulated within oil-in-water nanoemulsions. Measurements were made before and after the micelle phase samples were filtered. Reproduced with permission from Elsevier (66)

which subsequent to peroral delivery gets emulsified in the aqueous component in the gastrointestinal tract. The surfactants used should be ionic or non-ionic and must have HLB between 9 and 18 and more preferably between 11 and 16 (67). The advanced version of the self-emulsifying system was also developed, called as solid SEDDS (S-SEDDS). S-SEDDS can be easily developed by admixing them with inert excipients commonly used in granule formation (68). In 2014, Jie *et al.* formulated a solid self-emulsifying drug delivery system of vitamin D by spray-drying technology and investigated for bioavailability (BA) and anti-inflammatory activity. Treatment with formulation reduced myelo-peroxidase (MPO) activity, oxidative stress, C_3 protein level and O_2 level (69). Calcitriol, an active vitamin D analogue, is highly prone for chemical degradation in solid form; so, the development of its self-emulsifying drug delivery system would be useful. In this direction, a soft gelatin capsule of calcitriol was developed by first preparing the calcitriol solution and then encapsulating into soft gelatin capsules. The calcitriol solution was prepared by dissolving calcitriol in oil base. The calcitriol soft gelatin capsule was finally filled into the hard gelatin capsule pre-filled with granules of active absorbable algal calcium and zinc sulphate (70).

Nanoparticles

Nanoencapsulation of the bioactive is an efficient way to overcome the limitations of low bioavailability and storage degradation. This approach possesses the potential to increase bioactive solubility, enhance release behaviour and cellular uptake and prevent permeation of moisture and exposure to light. Selection of a nanocarrier and technique of formulation development is an important consideration to obtain optimum product (71). Biodegradability and biocompatibility are two essential parameters for selection of polymers (72). Recently, Milad Fathi *et al.* have formulated nanoparticles of vitamin D_3 using starch as a carrier material. Vitamin D_3, being hydrophobic, gets encapsulated inside a central hydrophobic core interconnected by amorphous regions of the polysaccharide units. The optimized batch composed of 2% *w*/*w* starch and 1.25% *w*/*w* vitamin D_3 obtained using ultrasonication (450w). The batch was observed with a particle size, polydispersity index and zeta potential of 23 nm, 0.42, and −37.36 mV, respectively. This formulation exhibited encapsulation efficiency in the order of 78%. The stability of the particle is inversely related to the size of the particle; so, the lower the size, the slower will be the Brownian motion and the greater would be the stability. The FTIR data had shown the interaction through hydrogen bonding between D_3 and starch, and this could be the reason for higher entrapment. The release profile showed less than 3.5% drug released during the first 2 h in gastric condition while maximum release during the next 8 h in the simulated intestinal fluid was observed. The presence of polysaccharide chains in starch prevents hydrolysis in an acidic environment and proved to be beneficial for the bioactive with a maximum rate of degradation at such condition. Thus, starch nanoparticles are promising carriers for VD_3 (73). Quinones *et al.* have formulated a controlled release system of ergocalciferol using a conjugation of ergocalciferol hemisuccinate with glycol chitosan using water-soluble carbodiimide reaction. The system finally obtained a self-assembled carrier in an aqueous media having a particle size in the order of 279 nm. The characterization of conjugation was investigated by FTIR spectroscopy and proton NMR. The release data showed a continuous drug release during the initial 8 h (74).

In an interesting study, Stenzel *et al.* have developed a controlled release system of 1,25 dihydroxyvitamin D_3 by formulating cross-linked microspheres prepared by polymerization using poly(vinyl neo decanoate-cross-linked-ethylene glycoldimethylacrylate) as a polymer. The release study showed 1% release of drug up to 40 days. The prepared microspheres were also non-toxic as confirmed by MTT assay and direct contact cytotoxicity assay (75). Subsequently, Wang *et al.* have developed a controlled release system of vitamin D_3 by encapsulating it inside the carboxymethyl chitosan (CMCS)-coated zein nanoparticles. Initially, nanoparticles using zein were developed, which were composed of cholecalciferol, and finally, a coating of CMCS was applied using calcium-assisted ion cross-linking method. The size of the particles developed was regularly monitored as increase in calcium concentration in any batch would result in an increase in the size of the particle. Vitamin D_3 coating with zein and CMCS provided better controlled release and improved photostability against UV light (76).

Recently, Vavia *et al.* formulated once-a-month delivery system of cholecalciferol using poly(lactic-*co*-glycolic acid) as sustained-release polymer. The concentration of the stabilizer was considered important for enhancing the entrapment as a decrease in the entrapment was observed in the absence of a stabilizer. The drug was observed to be amorphous in the polymer matrix as determined by DSC and XRD which in turn confirmed the ability of PLGA in improving the dissolution of cholecalciferol. The once-a-month drug release was also confirmed by the increase in half-life of the drug (77). The light-sensitive nature and poor dissolution of vitamin D are the two main problems which have been addressed in another excellent study. The formulation composed of 20,000 IU of vitamin D (0.25–1.0% *w*/*w*) and lipid-based carrier especially coconut oil or palm kernel or poloxamer 188, a combination of fatty acids such as Miglyol® 812N (98.95–99.7% *w*/*w*). This fatty acid is a mixture of caprylic/capric fatty acids which can easily solubilize fat-soluble vitamins and provides oxidative protection through its antioxidant properties. Butylated hydroxyanisole or butylated hydroxy toluene was also added as an antioxidant. The final formulation was then filled into a capsule made of a non-animal-derived polymer as HPMC. The formulation was then determined for dissolution and stability testing, and formulation containing poloxamer 188 or miglyol 812N/BHT showed an excellent stability profile with limited degradation following storage at ambient conditions after 10 weeks (78). Arachitol Nano™ is a commercially available nanoparticle-based formulation of vit. D_3 (60,000 IU/5 ml) as oral solution. This formulation was investigated for determining the absorption rate from the duodenum, jejunum and ileum using the rat intestinal sac technique. The increase in absorption through the various segments of the rat's small intestine, with high flux and permeability coefficient, was observed in comparison to conventional formulation. The commercial formulation was also observed to be stable at different pH, and no effect was observed on interaction with bile salts. The results showed that novel drug delivery system of vit. D_3 in

the form of Arachitol Nano™ is the best-suited formulation for oral administration of vitamin D (79).

Solid Dispersion

Solid dispersion (SD) can be obtained by dispersing a drug onto a solid carrier. As per surface morphology analysis by SEM, the final formulation appeared in the form of the amorphous structure (80). The pharmaceutical/nutraceutical preparations containing vitamin D can be prepared by solid dispersion technique. In a recent study, a dispersion of vitamin D was formed containing adherent as polyethylene glycol (MW 1500–8000 Da) by dissolving them in an organic solvent (*e.g.* ethanol) having low toxicity. The dispersion was also composed of an antioxidant and a chelating agent as metal extractor for chemical stability. Finally, spray dryer was used to spray vitamin D dispersion over calcium salt and granules were produced which were observed to increase the stability of vitamin D (81). The solid dispersion of vitamin D and bisphosphonate was developed using cyclodextrin as amorphous polymer along with stabilizing agent or pharmaceutically acceptable additives. The optimized formulation was obtained by preparing different formulations in the range of vitamin D and cyclodextrin weight ratio of 1:100 to 1:1600, respectively. Vitamin D was employed in an amount ranging from 0.01 to 10% by weight, while bisphosphonate in 1 to 30% weight range. The stabilizing agents were added to prevent oxidation, and pharmaceutical additives, including binding agent, lubricant, disintegrant, diluents, filler, compressing aid, buffer, suspending agent, emulsifying agent, surfactant and coloring agent, were also added. The stability testing of this formulation showed that there was no significant change in the content of vitamin D_3 till 4 weeks (82). In another study, a stabilized formulation of calcium and vitamin D (1–2 g of calcium for 500–1000 IU) was developed which leads to a high level of bio-availability using propylene glycol or polyethylene glycol (weight range of 300–1500), liquid paraffin or silicone oil. The even and diffused distribution of glycols (5–15% for 30–80% *w*/*w* of calcium) within the granulating mixture played a binding effect which in turn allowed even distribution of vitamin D and improved the flow properties of calcium (83). In another invention, a solid pharmaceutical preparation of the active form of vitamin D_3 was formed by developing two layers. Development of layers depends upon the solubility of the polymers in an organic solvent as the highly soluble polymers were composed of the external layer while the slightly soluble were composed of the inner layer. The inner layer was also composed of a basic substance which was added in concern of its stability in the acidic medium. Different additives, such as basic substances, were examined for neutralization capability, and among them, one with more basicity and low water content was selected. While conducting stability studies, it was observed that a new excipient with low water content and maximum absorption capability is present as the inner layer would limit the degradation of vitamin D_3 (84).

FUTURE PERSPECTIVE

A great deal of literature on epidemiological studies have observed the association of serum vitamin D and calcium levels with the degree and severity of multiple diseases, including autoimmune diseases, infectious diseases, type 1 and type 2 diabetes, inflammations, cancer, hypertension, multiple sclerosis, and thyroid and pulmonary diseases. However, the precise connection between vitamin D status and associated diseases is still unclear and even more clinical data is required to support such facts. Some studies have also suggested slight or no effect of serum vitamin D on various diseases. Such insignificant effect in the development and severity of many diseases may suggest a hypothesis that lowering serum concentration of vitamin D could be the result but not the etiological reason of ailments. So, serum vitamin D can be assumed as the biological marker of deteriorating health in response to the development of any disease. The therapeutic application of these insights is still at a preliminary stage, but the availability of new structural analogues of $1,25(OH)_2$ D_3 and those under pipeline with favorable *in vivo* profiles is well-documented, and it is likely that clinical trials will demonstrate their significance in developing a good relationship with associated diseases. However, rate-limited dissolution profile is common with all of the developed analogues, and even thermal degradation as well as light sensitivity issues are some factors which made the work of formulation scientists tough. Dissolution profile of a drug can be improved either by particle size reduction, cyclodextrin complexation, salt formation, emulsification, pH modification and amorphization. The salt formulation, micronization and pH modifications are conventional techniques of solubility enhancement, while techniques, such as solid dispersion, self-emulsifying drug delivery system and nanoemulsification, are generally identified as non-conventional techniques. In order to commercialize such products, a lot of investment is required in the field of manufacturing, including hot melt extruder, high-pressure homogenizer and rapid milling equipment. Considering bioavailability enhancement and stability improvement, the approaches of SEDDS, solid dispersion, solid lipid nanoparticles and nanoemulsification are favorable options. In conclusion, it is high time for pharmaceutical companies and research labs to start focusing on the ways to deal with vitamin D deficiency as there is a huge stratum of human diseases which etiologically depends upon vitamin D concentration in the blood. A judicial selection of drug delivery system and their excipients would finally lead to efficient formulation development for vitamin D in order to improve its stability and pharmacokinetic profile.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that there is no conflict of interest associated with this publication. The institute publication number for this manuscript is IIIM/2240/2018.

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