1. INTRODUCTION

Nutritional deficiencies and inadequacies have become a major health issue all across the globe. Deficiency in vitamin D, folate and iron are noted among adolescents, children, pregnant women and elderly (Hwalla *et al.*, 2017). Deficiency of vitamin D is one which always remains underdiagnosed due to false perceptions that few minutes in sunlight and regular diet provides sufficient amount of vitamin D (Gupta and Gupta 2014). 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 but unfortunately, this strategy has been failed till date as only a few fortified products are available in the market. Fortification, the only option which if utilized at large scale can become successful for providing 2000IU daily dose requirement and helps in reducing the menace of associated diseases (Kennel *et al.,* 2010).

1.1 Biological functions of vitamin D

Vitamin D is an essential micronutrient 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 bone so, deficiency of vitamin D is a major menace causing rickets, osteomalacia, hyperparathyroidism and osteoporosis (Thacher and Clarke, 2017; Mostafa and Hegazy, 2015). 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 (FAO/WHO 2001). Type 1 and Type 2 diabetes mellitus patients reported being in

jeopardy if vitamin D deficient, as chances of cardiovascular mortality, insulinemia and glucose intolerance increases (Lavie *et al.*, 2011; Joergensen *et al.*, 2010; Schwalfenberg G, 2008; Fig. 1.1). The efficiency of vitamin D analogues as an anticancer agent has also been reported due to its ability of inhibition of proliferation, differentiation and angiogenesis (Tuohimaa P, 2008; Garland *et al.*, 2009).

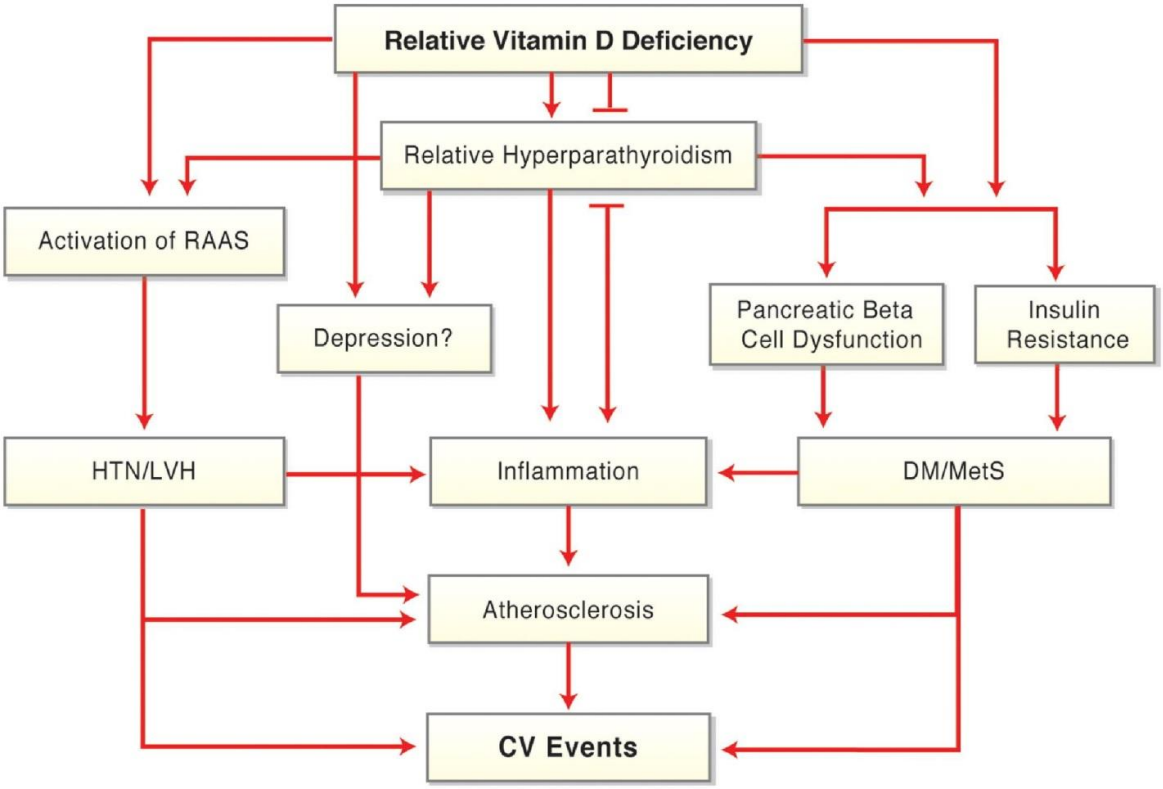

Figure 1.1: Cardiovascular events on vitamin D deficiency (Lavie *et al.*, 2011).

1.2 Recommended dietary intake of vitamin D

The serum level of 25-hydroxyvitamin D [25(OH)D] indicates vitamin D status and minimum required concentration is 20 ng/ml (Vitamin D: Fact sheet, 2017; Table 1.1). As per Institute of Medicine (US), a study on Germans (aged 20-70 years) 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 increased osteoid in men and women with a concentration of 20 ng/mL - 30 ng/mL of

25(OH)D in the blood. As per few reports, 34 ng/mL and 38 ng/mL of 25(OH)D is required for calcium absorption from the intestine and for performing the neuromuscular function so, 40 ng/mL was the final concentration which was decided to prevent hyperparathyroidism (Hollick M, 2013). The nutrient guidelines published by US and Canada depicting dietary requirement of vitamin D was presented in (Ross *et al*., 2011; Table 1.2).

Table 1.1 Concentration of 25(OH)D in blood with associated health impact*

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)

*From Vitamin D: Fact sheet, 2017

Table 1.2. Dietary reference intakes for vitamin D and calcium*

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 month	200	200	1000	400	400	1000
Infants 6 to 12 month	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

*Modified from Ross et al., 2011.

1.3 Risk factors

The sunshine vitamin requires regular exposure of 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. 1.2). Excessive use of sunscreen has become a problem since their composition is designed to filter UVB rays, which are essential for vitamin D production (Mcneill and Wesner, 2018; Clemens *et al.,* 1982). People with darker skin tone are more prone to less absorption of UVB light, thus necessitates higher exposure to produce an equal quantity of vitamin D as required by fairer skin tone (Holick MF 2007). 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 with low BMI value and so required larger vitamin D intake (Patients *et al.,* 2011). The high antagonistic effect was reported by the administration of phenobarbital and phenytoin on vitamin D associated effects as both of the drugs induces the rapid metabolism of vitamin D (Mazahery and von Hurst, 2015; Grey et al., 2005). The presence of severe condition of hyperparathyroidism and granuloma-forming disorders also serves rapid metabolism of vitamin D (Adams and Hewison, 2006). Breastfed infants are more susceptible to vitamin D deficiency and for this American Association of Pediatricians (AAP) recommended 400 IU of vitamin D per day to them (Wagner and Greer, 2008). Ageing also reduces the capacity of vitamin D synthesis due to a decrease in 7-dehydrocholesterol precursor in the skin (MacLaughlin and Holick, 1985).

1.4 Population under risk factor

Vitamin D deficiency is primarily noted among children, young person, pregnant women and elderly (Hwalla *et al.,* 2017). In the Indian subcontinent, 70%-100%

general population was observed to be affected by vitamin D deficiency and this rate is common in both rural and urban populations (Gupta and Gupta, 2014). The report published by 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 (Mithal *et al.*, 2009). Prevalence of deficiency with rate of 80.9% was observed in women with age over 80 years in European continent (Bruyere *et al.*, 2014). In American continent, its deficiency is prevalent in 42% of adult, 82% of black individuals and 69% of the Hispanic population (O'Keefe *et al.*, 2012).

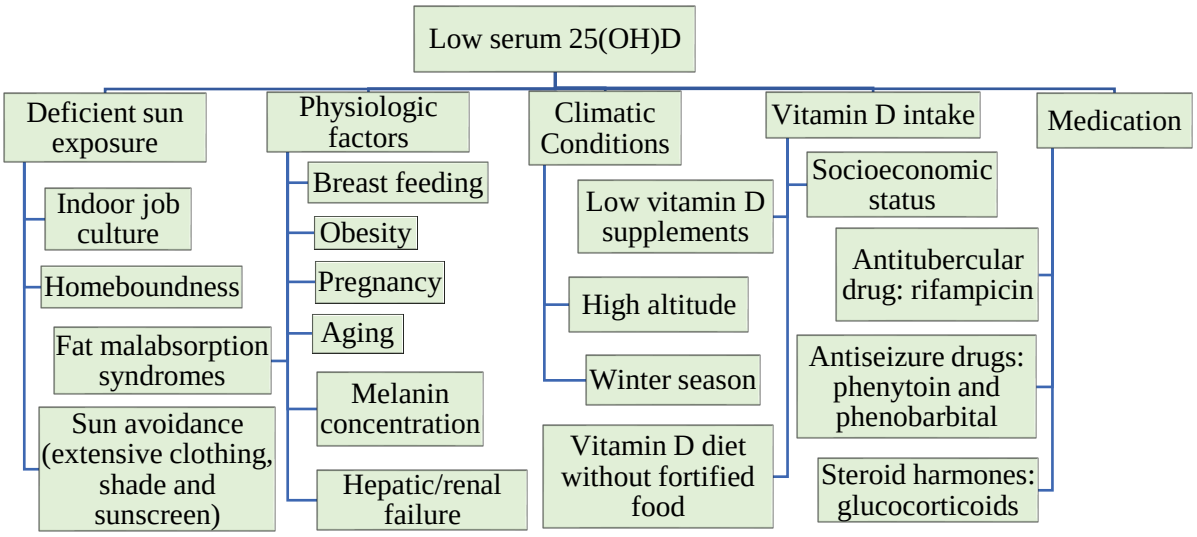

Figure 1.2: Risk factors associated with vitamin D deficiency (Patients *et al.*, 2011).

1.5 Vitamin D Compounds

Food products like mushrooms and fish are two main sources which are composed of vitamin D but only at very minute quantity. Even non-availability of sufficient fortified milk and fortified cereals have made the condition worse (O'Mahony et al., 2011). So dependence on supplements has become necessary. Vitamin D supplements are

available in form of ergocalciferol (vitamin D_2), cholecalciferol (vitamin D_3) and vitamin D analogues.

1.5.1 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 of UV irradiation to ergosterol (Japelt and Jakobsen, 2013). Vitamin D_2 is a fat-soluble vitamin which appears as odourless white crystals with a very poor solubility (0.05mg/ml). The stability of pure form of vitamin D_2 is also one of the biggest issues since it readily gets oxidized and inactivated in the moist atmosphere in the short span of few days. Ergocalciferol as such is inactive and needs two hydroxylation before the appearance of active form. The complex metabolic pathway of ergocalciferol involves initial production of ercalcidiol (25-hydroxyergocalciferol) on the exposure to an enzyme present in liver and final production into ercalcitriol (1,25-dihydroxyergocalciferol) in kidney. The plasma concentration of 25-hydroxyergocalciferol is considered by clinicians as an amount of vitamin D_2 in body while ercalcitriol (1,25-dihydroxyergocalciferol) is the active form of vitamin D_2 in body (National Center 2017; DeLuca HF, 1988). 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 (Holick *et al.*, 2007). 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 affinity for vitamin D

binding protein (Hollis BW, 1984; Houghton and Vieth, 2006; Cheng *et al.,* 2003; Cheng *et al.,* 2004). There is one report focusing on the production of an additional product 1,24,25$(OH)_3$D by 24-hydroxylation after 25-hydroxylation in 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 it still needs an extra side-chain oxidation for deactivation (Horst et al., 986). 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 6-week period (Heaney et al., 2011). Commercial multivitamins preparations may contain either vitamin D_2 or D_3, but there are emerging trends to formulate vitamin D product containing vitamin in the form of D3 (Table 1.3). The shift towards vegan is the new trend prevailing in world and India is the only country where 35% of people are strict vegetarians who do not consume animal products (Perry et al., 2001; Key et al., 2006). 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 ergocalciferol, a plant-based vitamin D, to completely eradicate the prevalence of vitamin D deficiency from the society.

1.5.2 Vitamin D_3 (Cholecalciferol)

Vitamin D_3 is an animal-based natural molecule produced as derivative of 7-dihydroxycholesterol on exposure of skin to UV radiations (Wacker and Holick, 2013). Fat-soluble nature reduces its solubility (0.013mg/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, calcifediol when reaches to the kidney with the help of hydroxylase produces an active form known as calcitriol (Zand and Kumar, 2017). The physicochemical properties like solubility, lipophilicity and dissociation constant of D_3 are almost similar to D_2 but the difference is only at metabolism and targeting.

Table 1.3. Study characteristics of ergocalciferol and cholecalciferol

Intervention, dose and frequency	Sex and 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 30y M 49.5y (n= 33)	12 wk	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 found 87% more potent at raising 25(OH)D.	Heaney et al., 2011.
a) Single oral dose of 300,000 IU vitamin D_3 b) Single IM 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 66-97y (n= 32)	60d	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., 2008.
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 20-61y (n= 30)	28d	28-d AUC was significantly greater for vitamin D_3 supplementation group than vitamin D_2 supplementation groups ($P < 0.002$).	Armans et al., 2004.
a) Ergocalciferol 1000 IU/d b) Cholecalciferol 1000 IU/d	(n= 70)	3mo	VitaminD_3 supplementation showed a 31% greater increase in serum level of 25(OH)D than was vitamin D_2 supplementation.	Glendenning et al., 2009.
a) Study 1: single IM 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 wk	Higher serum 25(OH)D level were obtained with vitamin D_3 intervention	Leventis& Kiely, 2009.

1.5.3 Vitamin D analogues

The establishment of knowledge that vitamin D hormones functions through a nuclear receptor and presence of these receptors into the tissues 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 (Levin *et al.,* 2007; DeLuca HF, 2005). The VDR activation led to classical (effects on endocrine system) and non-classical effects (on cardiovascular, renal, adaptive and innate immune system) (Plum and DeLuca, 2009; Cunningham and Zehnder, 2011; Gallieni *et al.,* 1995; Dusso *et al.,* 1991; Bouillon *et al.,* 2008). Development of analogs with minimum effects on mineral metabolism are desired since major proportion of serum 25(OH)D was utilized in mineral metabolism (Plum and DeLuca, 2010). Some vitamin D analogs 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 (Cunningham and Zehnder, 2011). Development of new analogs is distinguished as prodrug and active compounds. Ergocalciferol, Cholecalciferol, Alfacalcidol, Doxercalciferol and Calcifediol are prodrugs which require enzymatic activation while natural hormone Calcitriol and newly developed side chain modified products Paracalcitol, Maxacalcitrol, Oxaccalcitriol are already developed compounds (Table 1.4, Fig. 1.3).

Table 1.4. Vitamin D analogs and their physicochemical properties*

Chemical	Half life (hr)	pKa	Aqueous solubility (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-hydroxy vitamin 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-vitaminD_3)	–	–	Insoluble	–	Fulsatn, Hornel	Secondary hyperparathyroidism

*Pub Chem Compound Database (National Center for Biotechnology 2018).

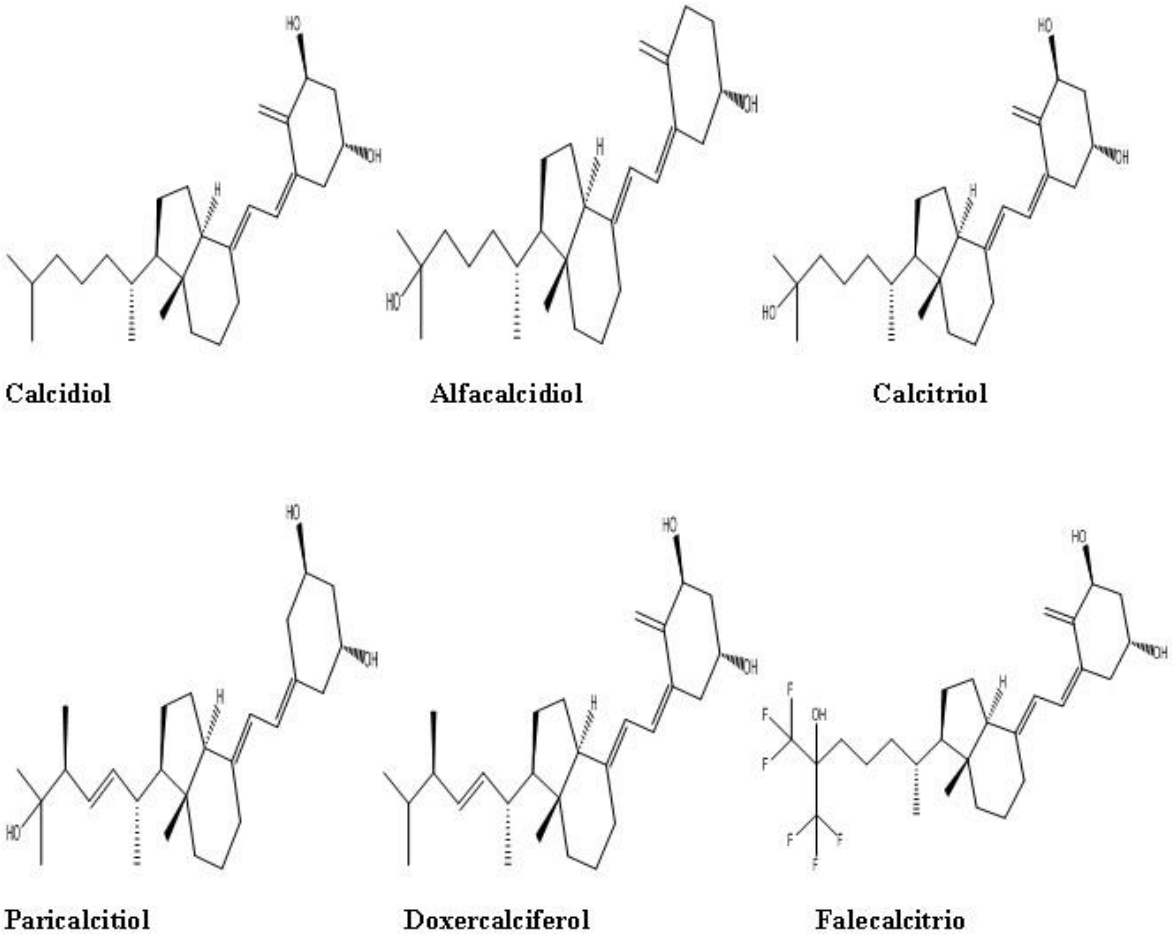

Figure 1.3: Structure of vitamin D analogues

1.6 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 single bond between C22 and C23 and absence of 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 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. The enrichment of food products with cholecalciferol or the development of cholecalciferol-based formulations is challenging because it is highly susceptible to degradation under environmental conditions including light, temperature and oxygen that can cause loss of its functionality and physiological benefits (Gonnet *et al.*, 2010). Moreover, it has been observed that the degradation rate of cholecalciferol is high in the low pH range, the rate decreases as pH rises, and the optimum pH for the stability was 6.5 to 8.0 (Makino and Suzuki, 1995). Keeping these aspects into consideration, novel formulation strategies are required for the efficient delivery of cholecalciferol (Gupta et al., 2019). Various delivery options have been explored to prevent the degradation of cholecalciferol including casein micelles (Haham *et al.*, 2012), zein nanoparticles coated with carboxymethyl chitosan (Gonnet *et al*., 2010), carboxymethyl chitosan-soy protein complex nanoparticles (Teng *et al.*, 2013), solid dispersion (Yuan et al., 2013), and microspheres (Diarrassouba *et al.*, 2015). 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. 1.4).

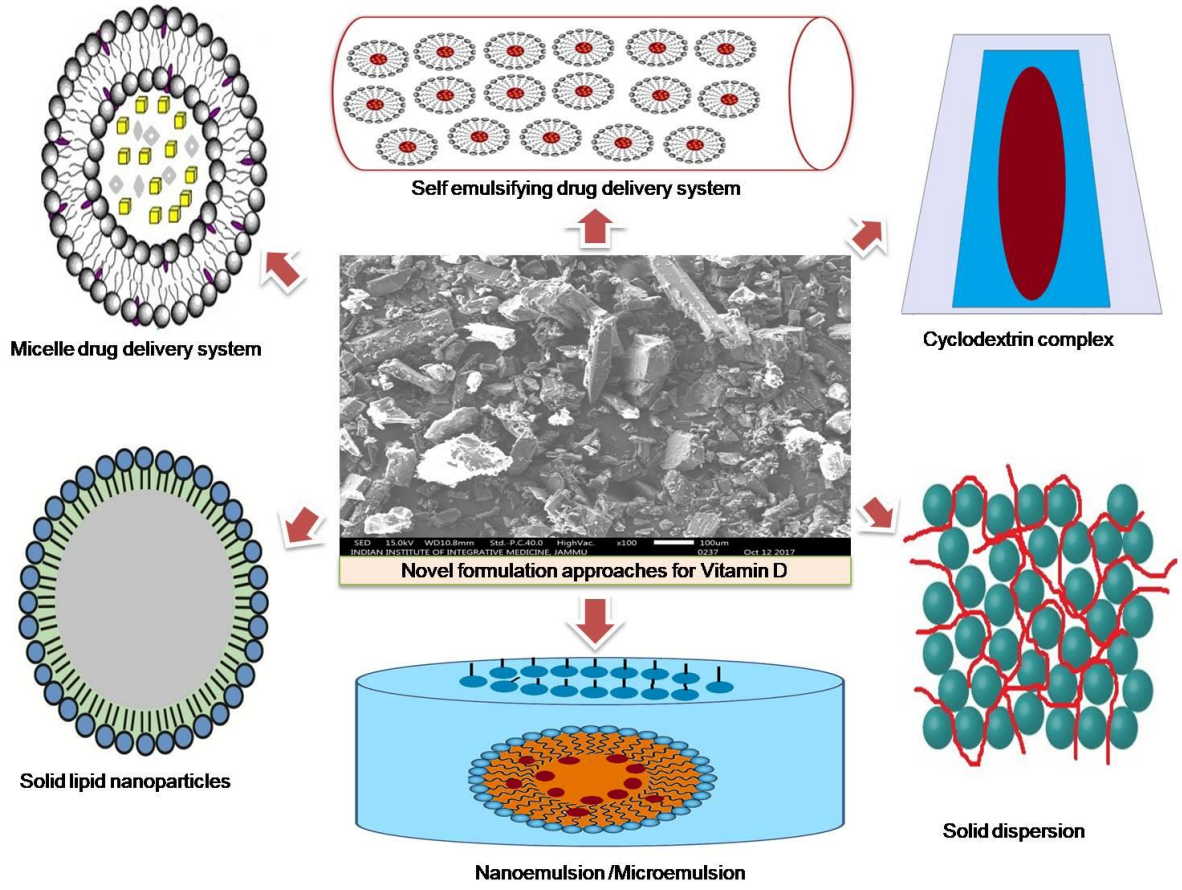

Figure 1.4: Formulation approaches for vitamin D

1.6.1 Solid Dispersion

Oral medication is the most ideal delivery route because of simplicity of administration, patient compliance and adaptability in medicines. However certain difficulty arises for oral route, for example, limited medication retention results in poor bioavailability and poor pharmacological action (Jain NK, 2004). Medication ingested from the gastrointestinal (GI) tract can be constrained by an assortment of factors with the most noteworthy being low solubility and bioavailability of the drug molecule. Following oral intake of drug, it should initially break down in gastric and intestinal liquids and after that it comes in blood circulation *via* various processes. Consequently, a

medication with low aqueous dissolvability will display dissolution rate limited absorption, and a medication with low membrane permeability will ordinarily display permeation rate limited absorption. Therefore, two strategies used to enhance the oral bioavailability of drug molecule include improving solubility and dissolution rate of inadequately water-soluble medications and upgrading the permeability of poorly permeable drugs. Various formulations containing solid dispersions have been illustrated in the previous report to increase the dissolution properties of drug with low aqueous solubility (Arunachalam *et al.*, 2010). Nearly 40% of new drug entity are associated with the limitation of poor aqueous solubility. In order to address the limitation of poor solubility and in turn-bioavailability, various delivery option have been attempted such as nanoparticles, solid dispersions and self-emulsifying system (Huda *et al.*, 2011). Solid dispersion received great attention to improve solubility and dissolution rate of drugs with low aqueous solubility. The term solid dispersion generally comprises a hydrophilic polymer and a hydrophobic drug. The physical state of polymer can be either amorphous or crystalline. In aqueous media, the carrier breaks down and the release of the colloidal particles of drug takes place. The subsequent increase in surface area produces significant increase in dissolution rate and improves bioavailability of inadequately water-soluble medications. Also, in solid dispersion, a portion of medication breaks up promptly following contact with gastrointestinal tract liquid to saturate it and this further result in precipitation of drug molecules as fine colloidal particles or oil globules of submicron size (Baghel *et al.*, 2011). Particularly for class II substances as indicated by the Biopharmaceutics Classification System (BCS), the bioavailability might be improved by increasing the solubility and rate of release during dissolution of the medication in the GI fluids (Leuner and Dressman,

2000). The solid dispersion technique has been widely utilized to address issue of poor dissolution and bioavailability of drugs with low aqueous solubility.

Chiou and Riegelman (1971) characterized the term solid dispersion as "a dispersion containing the eutectic blends of medications with water soluble carriers by melting of their physical blends". The term solid dispersion alludes to the dispersion of at least one active component fixing in an inert polymer at solid state arranged by melting (combination), solvent, or the melting solvent technique. It has been suggested that the medication was available in an eutectic blend in a crystalline state in solid dispersion (Sekiguchi and Obi, 1961), and subsequently it was stated that all medication in solid dispersion might not really be available in a crystalline state, a specific division of the medication may be in molecular dispersion in the polymer (Hancock and Zogra, 1997). In fluid media the polymer of solid dispersion broke up, leading to release of medication as fine, colloidal particles. With the associated increase in surface area, the dissolution rate and bioavailability of molecule with low solubility were found to be increased. The application of solid dispersion systems are limited due to certain manufacturing issues which can be overcome with the use of novel excipients including surface active agents. The clinical success of solid dispersion technology can be witnessed by various commercially available marketed products (Table 1.5) based on this technology (Sekiguchi and Obi, 1961; Noyes and Whitney, 1897; Das *et al*., 2011; Kumar *et al.*, 2011).

1.6.1.1 Advantages of solid dispersion

1.6.1.1.1 Particle size reduction: The solid dispersion containing hydrophilic carrier interacts with the aqueous medium, the hydrophilic polymer quickly breaks up leaving

the drug particles in a micro-fine state having massively expanded surface area and thus improved dissolution rate (Leuner and Dressman, 2000; Huang and Dai 2014).

1.6.1.1.2 Enhanced wettability: A noteworthy approach to enhance drug dissolution by the hydrophilic carrier is based on the wettability of drug offered by solid dispersions. The carriers which show surfactant activity like cholic acids enhance wettability. Nonetheless, carriers like urea that are without any surfactant properties were appeared to enhance drug wettability (Vasconcelos et al., 2007).

Table 1.5: Solid dispersion based marketed products.

Drug name	Brand name	Composition	Dosage form	Manufacturing company name
Nabilone	Cesamet	PVP	Tablet	Eli lilly, UK
Lopinavir	Keletra	PVPVA	Tablet	Abbott, USA
Griseofulvin	Grispeg	PEG	Tablet	Pendinal Pharm Inc, USA
Troglitazone	Rezulinb	HPMC	Tablet	Pfizer, USA
Nifedipine	Afeditab	Poloxamer/PVP	Tablet	Elan Corp, Ireland
Everolimus	Certican	HPMC	Tablet	Novartis, Switzerland
Fenofibrate	Fenoglide	PEG	Tablet	Life Cycle, Denmark
Etravirine	Intelence	HPMC	Tablet	Tibotec, USA
Verapamil	Isoptin SRE-240	HPMC/HPC	Tablet	Soliqs, USA

1.6.1.1.3 Higher porosity of particles: Carrier properties additionally impact the porosity as linear polymers deliver bigger and more permeable particles than reticulated polymers which results in enhanced dissolution rate. Expanded porosity permits better entrance of the dissolution liquid and additionally expanded surface region between the solid dispersion and dissolution medium that enhances drug dissolution (Vasconcelos *et al.,* 2007).

1.6.1.1.4 Drug in amorphous state: Drugs in this state are known to have more prominent fluid solubility than their crystalline counterpart. This is due to the arbitrarily condition of the amorphous drug that requires less vitality to break up contrasted with crystalline drug particles that require extra vitality to overcome the crystal lattice (Vasconcelos *et al.*, 2007).

1.6.1.2 Classifications of solid dispersion

The solid dispersions are classified into two categories: crystalline solid dispersion or amorphous solid dispersion which is based upon the physical state of the carrier. The carriers affect the properties of solid dispersion. Based on the hydrophilic carriers employed solid dispersions can be classified as shown in Figure 1.5 (Chiou and Rielman, 1971; Bhatnagar et al., 2014; Vo et al., 2013).

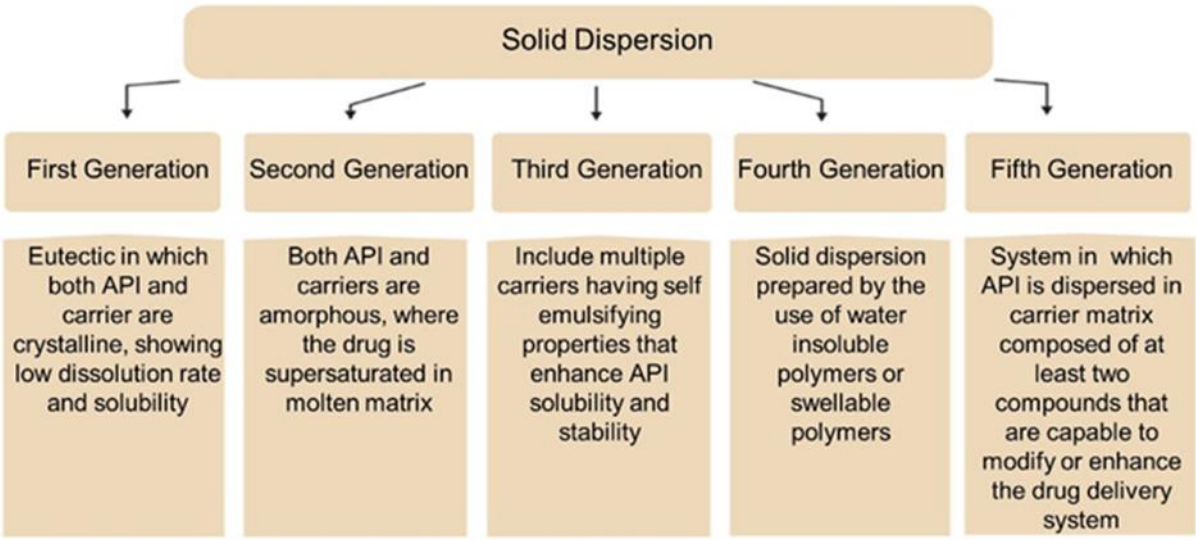

Figure 1.5: Classification of solid dispersion.

1.6.1.2.1 First generation of solid dispersion: It has been noticed that the eutectic blends of drug and carrier enhances the rate of drug release and thus, the bioavailability of poorly water-soluble medications. Around the same time, few solid dispersions were developed, for example, sulfathiazole (Sekiguchi and Obi, 1961) and chloramphenicol

(Sekiguchi and Obi, 1964) utilizing urea as hydrophilic carrier. These solid dispersions offered quicker release and increased bioavailability than other formulations. The better wettability of the drug and reduced particle size were the main explanations resulting in increased bioavailability. Afterward, Levy and Kaning developed solid solutions comprises of mannitol with enhanced dissolution because of better release from microcrystals (Levy G, 1963; Kaning JL, 1964) or particles (Simonelli et al., 1969). Mostly crystalline carriers were used in first generation of solid dispersions. The major carriers used in solid dispersions having crystalline nature incorporate urea (Sekiguchi and Obi, 1961; Sekiguchi and Obi, 1964) and sugars (Kaning JL, 1964). The major drawback of first-generation solid dispersion was limited drug release.

1.6.1.2.2 Second generation of solid dispersion: In the late sixties it was seen that solid dispersions prepared were thermodynamically stable but with poor rate of release of drug (Simonelli *et al.,* 1969; Chiou and Riegelman, 1969; Urbanetz NA, 2006). In the second generation of solid dispersions mostly amorphous carriers were used which are polymeric in nature (Vilhelmsen et al., 2005). The use of polymeric carriers results in era of amorphous solid dispersions. These were isolated into completely synthetic polymers and natural product-based polymers. Completely synthetic polymers incorporate povidone (PVP) (Karavas *et al.,* 2006; Drooge *et al.,* 2006; Pokharkar et al., 2006; Hasegawa *et al.,* 2005; Lloyd *et al.,* 1999; Yoshihashi *et al.,* 2006), polyethylene glycols (PEG) (Urbanetz, NA 2006; Guyot *et al.,* 1995; Yao *et al.,* 2005; Chiou and Riegelman, 1970) and polymethacrylates (Ceballos et al., 2005; Huang et al., 2006). Natural product-based polymers are basically made by cellulose derivatives, eg, hydroxypropylmethylcellulose (HPMC), ethylcellulose (EC) or hydroxypropylcellulose (HPC) (Tanaka *et al.,* 2006) or starch derivatives, as cyclodextrins (Tanaka *et al.,* 2006; Won *et al.,* 2005; Konno *et al.,* 2006; Verreck *et al.,*

2006; Rodier *et al*., 2005; Garcia *et al.,* 2006). The characterization of amorphous solid dispersions was done to investigate the physical interaction between drug and polymers (Lloyd *et al.,* 1999). The amorphous solid dispersion formed the homogenous mixtures of drug and polymer (Drooge *et al.,* 2006). In these systems, the true solutions were formed as a result of drug incorporation in a polymeric carrier (Vanden et al., 2006). Amorphous solid suspensions exist when the medication has restricted carrier solubility or a higher melting point (Chiou and Rielman, 1971). Molecularly, the obtained dispersion does not have a homogeneous structure. The final product obtained was amorphous when drug particles were dispersed in polymeric carrier. If the carrier contains the drug both in suspended as well as dissolved form then a heterogeneous structure is acquired with blended properties of amorphous solid solutions as well as of amorphous solid suspensions (Drooge *et al.,* 2006; Goldberg *et al.,* 1966). In second generation solid dispersions, the size of the drug particle was reduced due to the solubilization in the carrier and the drug exists in supersaturated state at molecular with better wettability and dispersibility (Won *et al*., 2005; Karata *et al.,* 2005; Damian *et al.,* 2000). In these solid dispersions, the drug release profile depends upon the dissolution of carrier.

1.6.1.2.3 Third generation solid dispersions: In this generation of solid dispersion the surfactants and the self-emulsifying carriers used for solid dispersion preparations. In these solid dispersions there was less chance of recrystallization and as a result better stability was observed. The utilization of surfactants, for example, inulin (Drooge *et al.,* 2006), inutec SP1 (Vanden *et al.,* 2006), compritol 888 ATO (Li *et al*., 2006), gelucire 44/14 (Yuksel *et al.,* 2003) and poloxamer-407 (Chauhan *et al*., 2005) as carriers was appeared to be successful due to better polymorphic activity and improved bioavailability. Further, the solid dispersion containing PEG and polysorbate 80 blend

of a poorly water-soluble drug LAB68 showed subsequent increase in the dissolution rate which further enhances the bioavailability of the LAB68 significantly by 10 folds in contrast with the dry mix of micronized drug (LAB68) and the stability was also increased (Dannenfelser *et al.,* 2004). The felodipine solid dispersion prepared with the use of blend of HPMC and poloxamer in polyoxyethylene hydrogenated castor oil (Rodier *et al*., 2005). The use of polymers and surfactants in the solid dispersion prevent precipitation as well as secure a fine crystalline precipitate from agglomeration into considerably bigger particles (Hoerter and Dressman, 1994).

1.6.1.2.4 Fourth generation of solid dispersion: These are the latest type of solid dispersions named as Controlled Release Solid Dispersions (CRSD). They contain medication with a short half-life and poor solubility which offer an extended release in a controlled way. Active pharmaceutical ingredient is dispersed in a carrier which enhances solubility while an insoluble swellable polymer may give extended release. They might be released by diffusion or erosion. Hydroxypropylmethylcellulose phthalate (HPMCP-55) and Aerosol were utilized as dispersing components though Eudragit RS PO and EC were used to impede drug release (Huang et al., 2006). EC can hinder drug release from solid dispersion**.** The various others carriers used in fourth generation of solid dispersions are HPC, HPMC, sodium carboxymethylcellulose (Na-CMC), and caboxyvinyl polymer (Carbopol). The CRSD mostly have same applications as of sustained drug delivery systems which include better patient compliance, reduced dosage frequency, prolonged therapeutic effect and fewer side effects (Vo *et al.,* 2013). The solid dispersion of aceclofenac prepared using polymer Gelucire 44/14, poloxamer 407, polyethylene oxide (PEO) showed increased dissolution profile of the drug with the use of surfactant and the zero-order drug release pattern due to the PEO which has water swellable property (Vo *et al.,* 2013).

1.6.1.3 Selection of suitable carriers for solid dispersion

The motivation behind incorporation of carriers in solid dispersion is to help in enhancing the dissolution rate of the medication through different mechanisms like enhanced wetting, solubilizing, and ability to stabilize amorphous medication against water-actuated crystallization (Craig DQM 2002; Konno et al., 2008). Different mechanisms credited to the inhibitory impacts of polymers against crystallization incorporate anti-plasticization by the polymers, interactions between the drug and polymers in solid dispersions, decrease in neighborhood molecular portability due to coupling between the polymer and API movements and an expansion in the activation energy for nucleation (Esfandyari et al., 2015). Carriers that are usually utilized in solid dispersion have been given in Table 1.6.

Table 1.6: Types of carriers used for solid dispersion.

Carriers	**Examples**
Surface active carriers	Gelucire 44/14, Compritol, Precirol
Natural product-based polymers	Cyclodextrins, Poloxamer-188, Poloxamer,407, Vitamin E TPGS, CMC
Small molecules	Urea
Emulsifiers	Tween 80, SLS, Sodium dodecyl sulfate, Bile salts
Sugars, polyols and their polymers	Mannitol, Sorbitol, Chitosan
Fully synthetic polymers	Polyacrylates, Polymethacrylates, HPMC, HPMCAS, HPC
Organic acids and derivatives	Succinic acid, Nicotinamide, Citric acid, PVA, PVP, PEG

1.6.1.4 Mechanism of drug release from solid dispersion

In solid dispersion the drug is dispersed in the carrier so that it can dissolve prior to the drug in the gastrointestinal fluid. The orally taken solid dispersion get saturated in the

GI fluid thereby increase in the drug bioavailability. There are mainly two mechanisms for the drug release (Fig. 1.6) which are described below:

1.6.1.4.1 Drug diffusion: This mechanism takes place in two steps. Firstly, the water is absorbed at surface of formulation which leads to the formation of either a carrier or gel layer around solid dispersion. The thickness as well as viscosity of these layers has a great impact on the diffusion of drug both in the bulk and across the layer. Secondly, the diffusion of drug across the solid dispersion layer takes place which is dependent on certain properties like drug solubility, particle size and polymorphic state of drug (Lloyd *et al*., 1999; Hallouard *et al.,* 2016).

1.6.1.4.2 Carrier controlled release: In this mechanism there is burst release of drug due to the fast dissolution of the carrier. It is further divided into two mechanisms based on the dissolution rate of the carrier: slow carrier dissolution and carrier erosion. In slow carrier dissolution the drug diffusion process takes place. However, solid dispersion erosion takes place when the drug and the carrier exist as separate particles where as if both the carrier and drug are dispersed well in the solid dispersion then the diffusion of the drug take place in carrier-controlled release (Hallouard *et al.,* 2016; Karavas et al., 2006). The ratio of drug and carrier was correlated to the dissolution profile of solid dispersion in various previous works (Okonogi *et al.,* 1997). Some publication also demonstrated that increase of carrier proportion leads to the decrease in the dissolution rate of drug (Kolasinac *et al.,* 2012). If the drug content is high then the erosion occurred where as if the quantity of drug is low then the diffusion mechanism takes place. Moreover, the carrier properties like solubility, viscosity, gel forming ability, polymorphic state and drug carrier ratio also have massive effect on the dissolution profiles of solid dispersion.

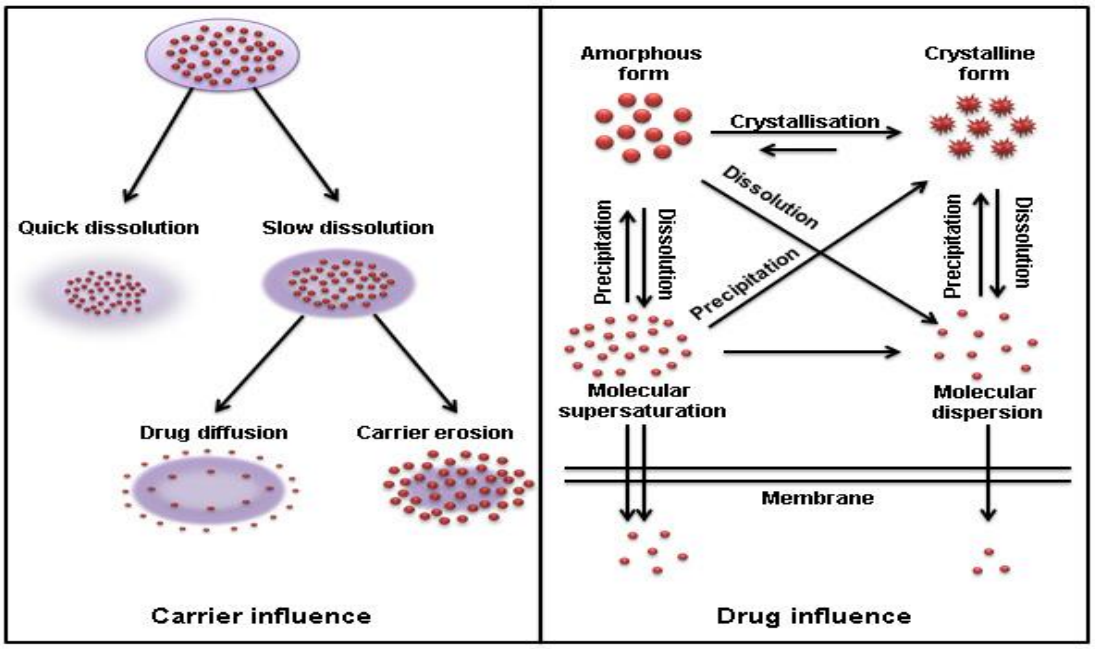

Figure 1.6: Mechanism of drug release in solid dispersion.

1.6.1.5. Recrystallization inhibitors as excipients in solid dispersion

The occurrence of recrystallization can be minimized with the proper selection of suitable polymers. For example, the solid dispersion of nimodipine and Povidone K-17 showed less recrystallization than that of solid dispersion of drug with PEG 2000 (Smikalla and Urbanetz 2007). The recrystallization inhibitors involve the mechanism of antiplastcizing effect *i.e.* the raising of glass transition temperature but there may be existence of some other mechanism which can inhibit the recrystallization. It was reported by Yoshioka et al, that recrystallization of indomethacin was prevented by 5% PVP concentration at high temperatures whereas less significant inhibition was occurred with higher concentration and low temperature. This clarify that there may be some other mechanism other than antiplastcizing effect of recrystallization inhibitors which can also be due to the specific steric and chemical interactions (Yoshioka *et al.*, 1995). The recrystallization inhibitors also enhance the stability of the formulations.

The solid dispersion of valdecoxib with PVP K30 retains the medication in amorphous system for a more period of time than that of HPC (Ambike *et al.,* 2004). Sertsou *et al.* described the prevention of crystallization of drug by HPMCP in solid solution (Sertsou *et al.,* 2002). During the conversion of crystalline form to amorphous the hydrogen bonds formed but with time the reverse was happened and recrystallization can take place which was only because of crystal growth formation from the nuclei. The development of nuclei takes place due to impurities or alpha relaxation during entropy change in the process of breakdown of hydrogen bonds again (Gunawan *et al.,* 2006). Various methodologies can be utilized for stability to forestall recrystallization of the components: First, the glass transition temperature of the solid dispersion should be raised. Second, molecular interactions between the medication and polymer additionally prompt stability (Miyazaki *et al.,* 2004). The stability of itraconazole was enhanced with the use of blend of Eudragit E100 and Polyvinylpyrrolidone vinyl acetate 64 in solid dispersion (Karel *et al.,* 2003). Shibata *et al.,* (2005) reported that the solid dispersion of drug with crospovidone enhanced the stability due to the hydrogen bond formation between crospovidone and drug whereas with no hydrogen bonding in the solid dispersion the amorphous state did not last longer which results in lesser stability. In another examination by Dhumal *et al.,* (2007) carrageenan was utilized with PVP in celecoxib solid dispersion and it was observed that the stability of solid dispersion was increased due to the carrageenan which keeps the celecoxib in amorphous form for longer period of time.

1.6.1.6 Methods for the preparation of solid dispersion

Solid dispersion technology is witnessing a number of innovations in the area of excipient being used and the manufacturing processes. This technology has tremendous

potential and a number of methods are available for the preparation of solid dispersion (Fig. 1.7).

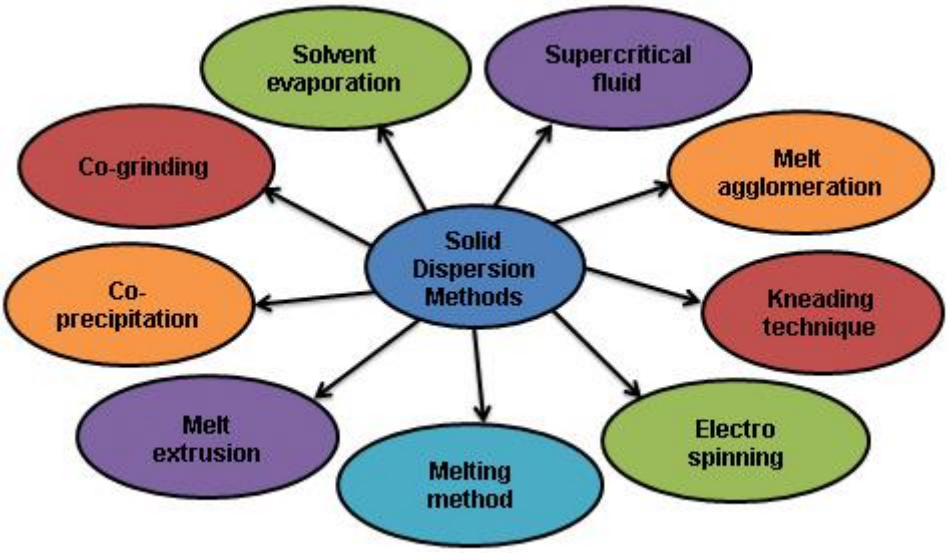

Figure 1.7: Various methods of preparation of solid dispersion.

1.6.1.6.1 Kneading technique: In this technique, the solid mass is obtained by the addition of water to the carrier and drug is kneaded for specific time. The plied blend is dried and subsequently passed through strainer to get solid dispersion with uniform size (Adley *et al*., 2008). This method was used for the development of valdecoxib solid dispersion with improved dissolution of drug (Modi and Tayade 2006).

1.6.1.6.2 Melting technique: In this method, drug and carrier are blended legitimately using mortar/pestle to get a homogenous mixture and subsequently blend is heated above the melting point of drug and polymeric. After that the blend is cooled to form a mass which is pulverized and sieved to acquire uniform dispersion. This technique does not involve use of any solvent and the homogenous mixture is obtained in molten state (Leuner and Dressman, 2000). However, this technique is linked with some

disadvantages as it is not applicable for thermo labile medications and high melting point polymer.

1.6.1.6.3 Co-precipitation technique: In this technique, the carrier is dissolved in suitable solvent and the drug is added to it and kept on magnetic stirrer for proper blending. An antisolvent is added further to promote precipitation and the precipitate is expelled, sifted and dried. The co-precipitation technique is an alternative technique for preparation of solid dispersion where desired physicochemical and biopharmaceutical characteristics are required (Mann *et al.,* 2018).

1.6.1.6.4 Co-grinding strategy: In this method, the drug and carrier are blended together and crushed in the assembly of a vibration ball. The solid crushing prompts the disfigurement of crystal lattice leading to decrease in crystallinity of medication and result in increased dissolution and bioavailability (Yamamoto *et al.,* 1974).

1.6.1.6.5 Solvent evaporation technique: In this method, the medication and polymer are dissolved in organic solvent followed by evaporation. The obtained mass is subsequently pulverized, sieved and dried. The product feature is associated with the nature of organic solvent and temperature for its removal via evaporation. Spray drying method is the upgraded method of evaporation technique wherein medication and polymers are broken down in a vehicle and subjecting it to hot air stream in the spry dryer. The solvent removal process has great impact on the product characteristics. Indeed, even the small amount of organic solvent used is ought to be evaporated so that the toxicity does not arise due to the higher amount of solvent in the formulation (Leuner and Dressman, 2000). Solid dispersions of β-lapachone were prepared by solvent evaporation method using PEG and PVP and the dispersions containing PVP showed better dissolution profile of drug (Dhirendra *et al.,* 2009).

1.6.1.6.6 Electrostatic spinning technique: In this technique solid strands of submicron widths are shaped by subjecting the polymeric arrangement or liquefy through a nozzle of millimeter size. With the evaporation of solvent, the solid strands of fibres are shaped, which can be gathered on a spinning mandrel. This method is suitable for the fabrication of nanofibres (Dhirendra *et al.,* 2009). Itraconazole solid dispersions were prepared using this technique and in comparison to other techniques like spray drying and film casting, the electrostatic spinning technique has more productivity and was flexible for industry scale up (Nagy *et al.,* 2016).

1.6.1.6.7 Melt extrusion strategy: This method involves the twin screw extruder through which medication and bearer blend is extruded and hence the dispersion is obtained. The speed of screw and moisture content are two important factor affecting characteristics of solid dispersions (Choksi and Zia, 2004). The solid dispersion of oleanolic acid was prepared by hot melt extrusion technique which enhanced its dissolution rate and bioavailability (Gao *et al.,* 2017).

1.6.1.7 Characterization of solid dispersion

The solid dispersions are characterized to detect the crystallinity in it which can alter the drug solubility as well as bioavailability. The various techniques for characterization of solid dispersion are given below (Giri and Kumar, 2012).

1.6.1.7.1 Thermal analysis techniques: In this technique, physical property of a substance is estimated as a component of temperature under a controlled temperature program. In differential thermal investigation, the temperature distinction that creates between an idle reference material and the sample is estimated, when both are subjected to an indistinguishable heating condition. It comprises of differential scanning calorimetry (DSC), dissolution calorimetry and isothermal microcalorimetry. These

techniques are used to determine the parameters like drug carrier miscibility, drug carrier interactions and stability of solid dispersion (Giri and Kumar, 2012). Drug carrier miscibility is one of the important parameters for determining stability since the addition of excipients can be considered as an effective crystallization inhibitor only if present in a single phase with a drug molecule. Among various techniques, DSC is usually considered as "gold standard" technique for determining miscibility of API within polymer blend which can be detected by the presence of single or multiple Tg events. Lu and Zografi have used DSC technique for determining miscibility of indomethacin within polymer blend of citric acid and observed single Tg within the mixture up to a weight fraction of 0.25 citric acid whereas an increase in citric acid concentration showed a second Tg event. Presence of two Tg event determined through DSC has made them concluded that amorphous-amorphous phase separation could occur on increasing citric acid concentration above 0.25 weight fraction (Lu and Zografi, 1998).

1.6.1.7.2 X-ray crystallography: X-ray crystallography is a considerable technique for knowing the arrangement of atoms inside a crystal lattice, in which a light emission beams strikes on a crystal and diffracts into numerous particular headings. A crystallographer can deliver a three-dimensional photo of the density of electrons inside the crystal by analyzing the angles and intensities of these diffracted beams. From this electron density, the mean places of the molecules in the crystal can be resolved with their chemical bonds. X-ray crystallography includes X-ray diffraction (X-RD) and X-ray powder diffraction and the former is used mostly for the solid dispersion characterization (Giri and Kumar, 2012). Liu *et al.*, (2013) have used X-ray diffraction technique for determining the presence of crystals in extrudates of carbamazepine based solid dispersion prepared by a combination of kollidon (VA64), soluplus (SOL) and

eudragit (EPO). Samples were scanned over a 2Ɵ range of 3-40° with a step size of 0.02° and a step time of 0.3 s. XRD profiles of above solid dispersion showed no evidence of carbamazepine crystals within the extrudate while such results were observed to be inconsistent with DSC results since XRD has a low sensitivity of determining small crystals as compared to DSC.

1.6.1.7.3 Spectroscopy: Different spectroscopic techniques like infra-red and Raman spectroscopies are utilized to monitor the difference between vibrational energies of crystalline and amorphous forms and the presence of sharp vibrational bands indicates the existence of crystallinity in formulation (Verhoeven et al., 2008). FTIR can detect about 99% of crystallinity in pure material. The spectroscopic method provides an important information about drug -carrier interaction in solid dispersion (Qi et al., 2008). Paradkar *et al.,* (2004) developed curcumin-PVP solid dispersion and characterized it by IR, SEM, DSC and XRPD techniques. The occurrence of intermolecular hydrogen bonding was confirmed by FT-IR analysis as broadening of the peak in the region of 3600-3400cm^{-1} was observed in case of solid dispersion formulation while no such broadening was observed in FT-IR spectra of pure curcumin.

1.6.1.7.4 Dissolution studies: It is mainly carried out at physiological temperature by using type II USP dissolution apparatus. The dissolution apparatus is used to determine the dissolution profile of solid dispersion or tablet and also used to find out the rate release of pure drug, physical mixture and solid dispersion which gives idea about dissolution rate. The effect of different carrier and their composition on dissolution rate of solid dispersion is mainly characterized by this technique (Patidar *et al.,* 2010). Newman *et al.,* (2011) have summarized 40 research papers on the basis of their dissolution and bioavailability data. Finally, all amorphous dispersions were characterized as with improved bioavailability, lower bioavailability and similar

bioavailability on comparing with reference material. The whole comparative analysis of such study has also revealed various in vitro and in vivo variables that could influence the final results. Dissolution testing equipment, type of dissolution media with its volume and pH, sink or non-sink conditions, rate of agitation and particle size of dispersion are some of the variables important for in vivo characterization while the reference material used for bioavailability comparison, species of animal used and fasting versus fed conditions are some of the variables important for *in vivo* characterization.

1.6.1.7.5 Scanning electron microscopy: This is a visual technique in which the surface morphology of the solid dispersion is studied (Mididoddi and Repka, 2007; Ozkanet al., 2009). It can also be used to monitor the recrystallization process on the surface of formulation. The limitation of scanning electron microscope is that it is restricted to the surface morphology used to determine the physical structures of solid dispersions but still can predict the crystallinity or amorphous nature of the solid particles. Paradkar *et al.,* (2004) have observed flat broken needles of different size with well-developed edges on analyzing the SEM of pure curcumin but amorphous as well as spherical particles were observed in case of solid dispersion with PVP. The drug-polymer ratios affect the results as 1:1-1:3 drug-polymer ratios were observed having pin holes within the spheres while smooth concave depressions were observed at 1:5-1:10 drug-polymer ratios.

1.6.1.8 Solid dispersion of vitamin D: 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 also composed of 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 increased the stability of vitamin D (Mahmoud and Ebeed et al., 2013). The solid dispersion of vitamin D and bisphosphonate were 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. The vitamin D was employed in an amount ranging 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 (Jin et al., 2010). In another study, a stabilized formulation of calcium and vitamin D (1-2 g of calcium for 500-1000 I.U.) 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 also improved the flow properties of calcium (Valleri and Tosetti, 2006). In another invention a solid pharmaceutical preparation of active form of vitamin D_3 was formed by developing two layers. Development of layers depends upon solubility of polymers in organic solvent as highly soluble polymers were composed of external layer while slightly soluble composed of inner layer. The inner layer also composed of basic substance which was added in concern of its stability in acidic medium. Different additives 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 present as inner layer would limit the degradation of vitamin D_3 (Makino and Suzuki, 1992).

1.6.2 Solid lipid nanoparticles (SLNs)

SLNs are generally considered as 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 aids in controlled release of drug, drug targeting and increased drug bioavailability (Kaur and Verma MK, 2014). Patel *et al.*, 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. This vitamin loaded SLNs was beneficial for the formation of clear fortified solutions (Patel et al., 2012). Park *et al.*, (2017) have focused on the bioavailability enhancement of cholecalciferol by formulating nano-structured lipid carriers using high pressure homogenization technique. The solid lipid sustained release nanoparticles of fat-soluble vitamins especially vitamin D_3 or vitamin D_2 were prepared using micro emulsion technique. In this work, a solid lipid core containing entrapped retinoic acid / vitamin D_3 were 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^{o}C and the micro emulsion so formed was cooled down to form crystals of SLN. The

characterization of particles for sustained release profile confirmed the 100% release of drug after 7 days (Kaur and Verma MK, 2014). The efficiency of solid lipid nanoparticles for the delivery of vitamin D3 was further confirmed by Demirbilek et al., 2017, where beeswax-stearic acid blend formulation was used. The particles with size range of 30-60nm 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 immunocopatible and also non-cytotoxic to keratinocytes (HaCaT), endothelial (HUVEC) and fibroblast (L929) cell lines (Demirbilek *et al.*, 2017). 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 drug in plasma of rat. The pharmacokinetic profile was improved in terms of AUC by 5.4 times and 29.4 times for ATRA and vitamin D_3 respectively in comparison to plane drug (Kumar *et al.*,2014). The circulatory movement of lipid coated cholecalciferol in blood with its absorption phenomenon is depicted in Fig. 1.8.

1.6.3 Nano-emulsion

Recently the attention has been focused on nano-emulsions, submicron emulsions, micro emulsion, fine-disperse emulsions and mini emulsions for improved drug delivery. Based on clarity parameters nano-emulsions are termed as transparent, translucent and milky. Small size is the only factor which imparts long-term stability (Maali and Mosavian, 2013). Guttoff et al, have developed a nano-emulsion 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/surfactant into an aqueous solution. The smallest particle size droplets were obtained in the nano-emulsions using tween 80 as a surfactant as compared to tween 20, 40, 60 and 85, at stirring speed of 800 rpm. The nano-emulsions 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^{o}C (Guttoff *et al.,* 2015). The development of nano-emulsions with utmost stability preventing precipitation requires amphiphilic nature of 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 nano-formulation 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 nano-formulation depends upon the ability to associate with biological tissues (Mousa AS, 2015).

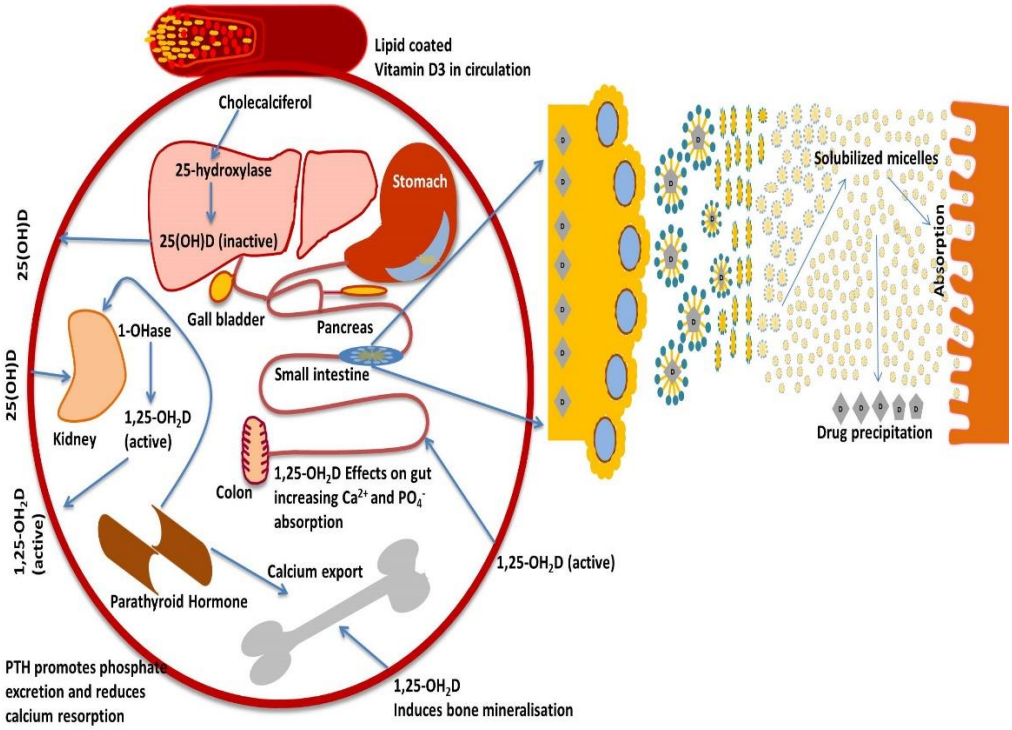

Figure 1.8: The oral administration of lipid based formulations interact with gastric lipase which initiates its digestion. Simultaneously, propulsion, grinding and retropulsion in stomach facilitate crude emulsion formation while in small intestine pancreatic lipase with its cofactor digests the lipids. The products of lipolysis usually in the form of mixed micelles transports into the epithelial cells where chylomicrons and very low density lipoprotein (VLDL) furthers enhances its absorption while some of drug molecules usually precipitated out after lipid digestion (Kalepu et al., 2013). Vitamin D_3 in circulation bound to its binding proteins and transport to the liver, the site where it gets converted to calcifediol (25-hydroxycholecalciferol). Finally, calcifediol when reaches to the kidney with the help of hydroxylase produces an active form known as calcitriol. Calcitriol in blood circulation effect small intestine for increased absorption of calcium and phosphate.

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 inner layer was applied directly to an inert core and exterior protective layer was applied directly to the interior layer. The inner core was 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 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 also composed of 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 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 antioxidant was in the range 0.4-0.9% w/w and the overall fixed ratio of cholecalciferol to anti-oxidant 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 does 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 was not more than 4% under standard accelerated conditions (40^{o}C & 75% RH for 3 months) or intermediate test conditions (30^{o}C & 65% RH for 12 months) (Fox and Shakib, 2010).

In another interesting investigation, Goncalves *et al.*, (2013), have estimated the effect of dietary fatty acids and their mixtures on the micellar properties of nano-emulsions 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 uptake was observed if mixtures of fatty acid were used. Finally, it was concluded that long-chain FAs, mainly poly-unsaturated free fatty acid (PUFAs) were less effective compared to other FAs particularly mono-unsaturated free fatty acid (MUFAs) in promoting cholecalciferol absorption but if a mixture of PUFA with other Fas were used the chances of cholecalciferol absorption would be increased. The extent of drug release from nano-emulsion 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 micelles is usually assessed as bioaccessibility, which is considered as the amount absorbed from biological membrane. Ozturk *et al.*, (2015), have

investigated the bioaccessibility of cholecalciferol. In this study, both long-chain triglycerides (LCT), as well as medium chain triglycerides (MCT), were used for nano-emulsion formulation. The amount of drug in the micelle phase was determined to be more in nano-emulsion composed of MCT as compare to LCT. It was concluded that the chain length of carrier oil is important factor for micelle formation rather than free fatty acid release.

1.6.4 Self-emulsifying drug delivery system (SEDDS)

This system consists of a mixture of active ingredient, oil, surfactant and co-surfactant 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 (Kohli et al., 2014). The advanced version of 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 (Boardman et al., 2006). Tang *et al.*, (2014), 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. Calcitriol, an active vitamin D analog 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 (Omrav et al., 2009).

1.6.5 Nanoparticles

Nano-encapsulation of bioactive is an efficient way to overcome the limitations of low bioavailability and storage degradation. This approach possesses the potential to increase bioactive solubility, enhances release behaviour, cellular uptake and prevents permeation of moisture and exposure to light. Selection of nano-carrier and technique of formulation development is an important consideration to obtain optimum product (Farokhzad and Langer, 2009). The biodegradability and biocompatibility are two essential parameters for selection of polymers (Wischke and Schwendeman, 2008). Recently, Hasanvand *et al.,* (2015) have formulated nanoparticles of vitamin D_3 using starch as a carrier material. Vitamin D_3 being hydrophobic gets encapsulated inside 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 23nm, 0.42, and -37.36 mV respectively. This formulation exhibited encapsulation efficiency in order of 78%. The stability of particle is inversely related to the size of the particle so lower the size slower will be Brownian motion and greater would be 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 2h in gastric condition while maximum release during next 8h 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 bioactive with a maximum rate of degradation at such condition. Thus, starch nanoparticles are promising carriers for VD_3. Quinones *et al.,* (2012), 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 initial 8h.

In an interesting study, Nguyen *et al.,* (2007), 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 upto 40 days. The prepared microspheres were also non-toxic as confirmed by MTT assay and direct contact cytotoxicity assay. Subsequently, Luo *et al.,* (2012), have developed a controlled release system of vitamin D_3 by encapsulating it inside carboxymethyl chitosan (CMCS) coated zein nanoparticles. Initially, nanoparticles using zein was developed which composed of cholecalciferol and then finally, a coating of CMCS was applied using calcium assisted ion cross-linking method. The size of particles developed was regularly monitored as increase in calcium concentration in any batch would results in an increase in the size of the particle. Vitamin D_3 coating with zein and CMCS provided better controlled release and improved photo stability against UV light.

Recently, Vora *et al*., (2017), formulated once a month delivery system of cholecalciferol using poly(lactic-*co*-glycolic acid) as sustained release polymer. The concentration of stabilizer was considered important for enhancing the entrapment as a decrease in entrapment was observed in 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 drug. 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 anti-oxidant properties. Butylated hydroxyanisole or butylated hydroxy toluene was also added as 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 an ambient conditions after 10-weeks (Huatan H, 2007). Arachitol Nano^{TM} is commercially available nanoparticle based formulation of vitamin D_3 (60,000IU/5ml) as oral solution. This formulation was investigated for determining the absorption rate from duodenum, jejunum and ileum respectively using rat intestinal sac technique. The increase in absorption through various segment of rat 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^{TM} is the best suited formulation for oral administration of vitamin D (Bothiraja et al., 2016).

1.7 Future perspective on vitamin D formulations

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, thyroid and pulmonary disease. Although the precise connection between vitamin D status and associated diseases is still unclear and even more of 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 finally 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 are still at 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 are 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 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 nano-emulsification 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 nano-emulsification 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 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 the stability and pharmacokinetic profile.