DEVELOPMENT AND IN-VITRO EVALUATION OF VITAMIN D3 NANO SUSPENSION

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Abstract
The present study framed the design and development of a nanosuspension of Vitamin D3 (VD3) for oral drug delivery. VD3 nanosuspension was prepared by oil-water emulsion process by using Eudragit polymer and poloxamer as a stabilizing agent. Its physicochemical properties were studied by Differential Scanning Calorimeter (DSC) and Fourier Transform Infrared Spectroscopy (FT-IR). The mean particle size of drug-loaded NPs obtained by a particle-sized analyzer was 92 to 200 nm. High encapsulation efficiency (>85%) showed that nanosuspensions were stable. In vitro, release study shows successful steady release of VD3 from nanosuspension. Almost 98% of VD3 was released from nanosuspension, which is 30% more than existing marketed formulations.

Keywords: Vitamin D3, Nanosuspension, Formulation, *In-vitro*, Polymer, Drug release

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INTRODUCTION

Vitamin D (VD) originated from a seco-steroid that resembles the structure of a cholesterol steroid (7-Dehydrocholesterol).[1] In human beings, VD exists in two different forms, such as D2 (ergocalciferol) and D3 (cholecalciferol).[2] VD2 is a dietary vitamin and readily available in the form of food substances such as cereals and salmon flesh, whereas VD3 is synthesized in the epidermis via sunlight interactions which constitute 95% of whole blood VD.[3] TheVD2 chemically differs from VD3 by possessing extra methyl functional group at C24 and as well as an extra double bond in between C22 and C23. These structural changes majorly influence the affinity of Vitamin D-Binding Protein (VDBP-52 KDa α Globulin protein) and their subsequent regulation in various biological functions in human beings.[4] Upon exposure to sunlight, the 7-Dehydrocholesterol undergoes photochemical cleavage between 9th and 10th carbon atoms of the steroid ring and forms to pre-vitamin D, which in turn undergoes the molecular rearrangement by the involvement of temperature to form Vitamin D. As per the literature, VD3 have three times higher affinity comparing to the VD2.[5] Thus it is evident that the active form of VD3-Calcitriol possesses significant role as a functional metabolite in various biological activities via genomic and nongenomic signaling pathways. Among the most reported functions of VD3, anti-microbial, anti-inflammatory, dysfunction of GAN (Giant axonal neuropathy) and acute lung injury are the most important.[6]In addition, the role of VD3 in the treatment of corona has also been reported.[7]

The serious threatening issue associated with VD3 is human intestinal absorption.[8]Several experiments have been conducted worldwide to elucidate the problems associated with human intestinal VD3 absorption. Majorly, patients with damaged small intestine due to chronic digestive and immune disorders, patients suffering from cystic fibrosis, and patients with high inflammation of the digestive tract (Crohn's disease) generally have low concentrations of VD3. In addition, people whose subcutaneous fat (BMI) is greater or higher than the required level, and patients undergoing radiation treatment are also known to contain fewer amounts of VD3.[9] Moreover, patients who are suffering from liver or kidney disease are also deficient with VD3.[10,11] The other important factors which strongly influence the levels of VD3 in the blood are lifestyle, pollution, drug malfunction, drug dumping, etc..

Maintenance of sufficient levels and subsequent proper functioning of VD3 in the body is a great challenge and is feasible by only external supplements. The use of different production methods and different formulation methods for the bulk production of VD3 worldwide is an essential task for various pharmaceutical companies and holds immense importance to combat VD3 deficiency disorders. In addition, most of the VD3 formulations (capsules, tablets, sachets) currently existing in the market are formulated based on fat-soluble formulationsbut not water-soluble.[12,13] As VD3 is a non-polar lipid, it exhibits poor solubility and low bioavailability in aqueous fluids. Therefore, the nanoformulations of VD3 with aqueous solubility are given priority.

The idea of this study was based on the limitations of modern vitamin D3 formulations that subsist in the market. Through the literature survey, we found that most of the pharmaceutical companies are producing Vitamin D3 in the form of oral formulations and soft gelatin capsules with 60,000IU of VD3.[12,14]These formulations have diminutive aqueous solubility followed by low bioavailability. Eventually,the above discussed, adverse effects have taken place. Hence, here we attempt to develop aqueous-based VD3 nanosuspensions and further investigate *in vitro* release of VD3 from these formulations which indirectly indicates drug bioavailability in the aqueousmedium. Further in the current research state of affairs, the drug delivery system with nano size is always an advanced technique where the drug is directly delivered to targeted cells.

EXPERIMENTAL

Material and Methods

Chemicals such as Vitamin D3, Eudragit RS100, butylated hydroxy anisole, polyoxyl 40 hydrogenated castor oil, Tween 20 and 80, EDTA disodium, propyl paraben, neotum, propylene glycol, glycerine, etc., are purchased from Sigma Aldrich, Mumbai. All the chemicals purchased were analytical grade.

Preparation of VD3 Nano formulation

VD3 nanosuspension has been prepared by oil in water emulsion (o/w) process. The Vitamin D3 (10mg) and Eudragit-100 polymer (RS or RL) were dissolved in 10 ml of methanol. This solution will act as an oil phase. This solution was homogenized for about 18-20 hrs at 30000 PSI. The water phase for the process was prepared by dissolving stabilizer poloxamer 407 in 20 ml of deionised water. This solution was incubated for 12 to 14 hrs. The oil phase was drop-wise injected into the water phase with high-speed stirring. The solution immediately converted into an emulsion of the drug and polymer. Formulations were prepared with varying polymer ratios overall four nanosuspension of VD3 **(Table-1)** were prepared. The prepared VD3 nanosuspension was evaluated by various analytical techniques.

Batch	Drug (mg)	Polymer (mg)		Surfactant Poloxamber 407 (%)	Distilled Water
		Eudragit RS100	Eudragit RL100		
VD3 – F1	10	8	-	1.0	20
VD3 – F2	10	16	-	1.0	20
VD3 – F3	10	-	8	1.0	20
VD3 – F4	10	-	16	1.0	20

Table 1: Nanosuspensions of VD3

RESULTS AND DISCUSSION

Differential scanning calorimetry (DSC) study was carried out to study the thermal behavior of the sample using DSC-60 (Shimadzu, Tokyo, Japan) calorimeter. DSC thermogramof VD3 nanosuspension showed a sharp peak at 87 ^{0}C **(Fig.1)**, which nearly, corresponds to the melting point of pure Vitamin D3. Further, the thermogram of other compounds was also nearby their respective melting points. It reveals that there was not any chemical interaction between VD3 and polymers.

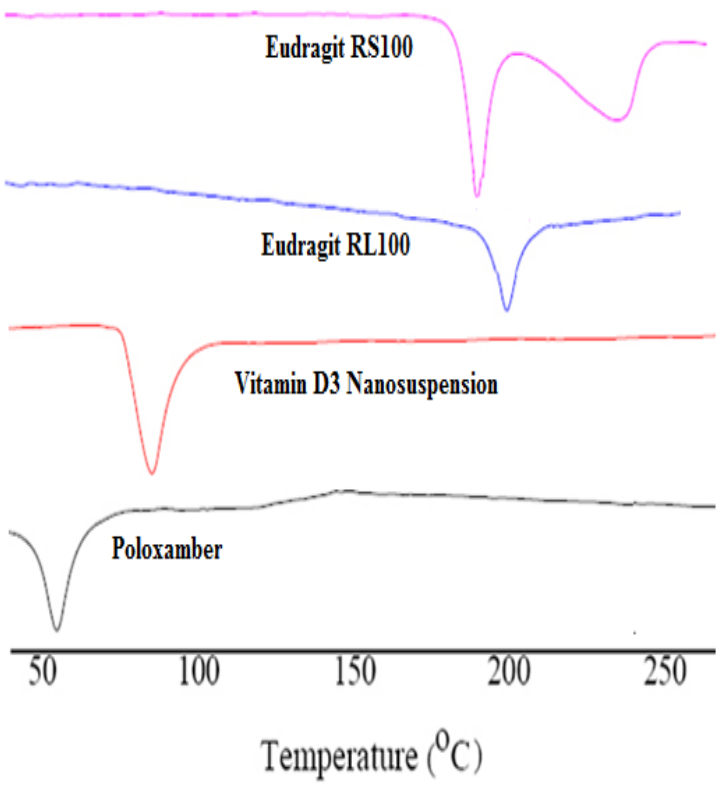

Figure 1: DSC Thermogram

The fundamental interaction between VD3 and Eudragit polymer was investigated by FT-IR. The spectra of VD3-nanosuspension shows almost all the characteristic peak like VD3-standard **(Fig.2a)**, which indicated there was no any kind of chemical interaction between VD3 and Eudragit polymer. It had been supported by the obtained specters of Eudragit-100-RL **(Fig.2b)** and Eudragit-100-RS **(Fig.2c)**.

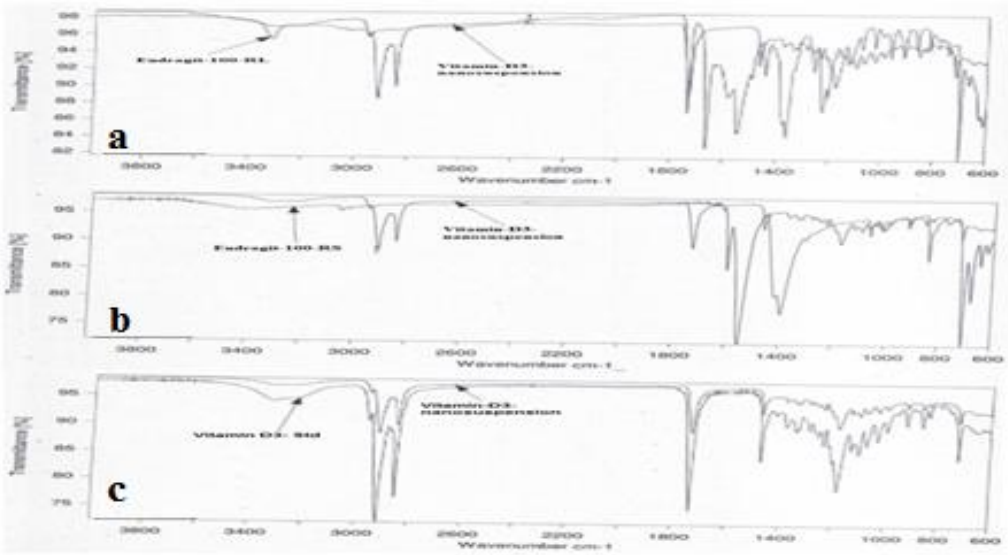

Figure 2: IR Spectra of (a) VD3 nanosuspension (b) Eudrgit-100-RL (c) Eudrgit-100-RS

The Particle size of the drug molecule is important because as the particle size reduces it can improve the dissolution of the poorly water-soluble drug. All the formulations of VD3 with a polymer

Eudragit RS100/ Eudragit RL100 with the Stabilizer Poloxamber 407 were determined for the particle size by dynamic light scattering (DLS) the result of the same is given in the table **(Table-2)**.

Drug	Particle size(nm)
VD3 – F1	200-240
VD3 – F2	189-200
VD3 – F3	135-180
VD3 – F4	92.20-105

Table 2: Particle size of VD3 nanosuspension

The drug entrapped in the VD3-nanosuspension was determined by the indirect HPLC method. The amount of free drug had been determined by HPLC after centrifugation of the aqueous suspension. The amount of encapsulated drug was determined as the result of the difference between initial drug concentrations and the determined free drug concentration. The encapsulation efficiency (EE %) could be achieved by the following equation.

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Total amount of drug loading- Free drug in the supernent}}{\text{Total amount of drug loading}} \text{ X 100}$$

The percentage encapsulation efficiency is an equally important parameter for the evaluation of formulation. The drug is entrapped inside it that will release slowly depending upon the polymer used. Eudragit RS100/RL100 releases the drug slowly and sustained from the polymeric nanosuspension these were determined HPLC. The entrapment of the formulation of VD3 is given in **(Table-3)** with the formulation code.

DrugFormulation	% DrugEntrapped	% Drug Unincorporated
VD3 – F1	80.21	19.79
VD3 – F2	83.68	16.32
VD3 – F3	85.10	14.90
VD3 – F4	91.21	8.79

Table 3: Drug entrapment efficiency

Saturation aqueous solubility of VD3 nanosuspension was also estimated against the VD3-Std drug. It has been found that the solubility of VD3-Std (72µg/ml) has low solubility in water it has been drastically increased in the case of VD3 nanosuspension. All four nanosuspensions have more aqueous solubility than VD3- Std**(Table-4)**.

Sr.No	Drug/Formulation Code	Saturation solubility (µg /mL)
1	VD3- Std	72 ±0.25
2	VD3 – F1	289±0.13
3	VD3 – F2	276±0.14
4	VD3 – F3	301±0.05
5	VD3 – F4	354±0.92

Table 4: Saturation Solubility of nanosuspensions

Cumulative drug release % of VD3 nanosuspension formulated with Eudragit RS100 / RL100

This study aims to develop steady-release nanosuspension of VD3. *In vitro* drug release profile of VD3 is shown in **Fig.3**. I*n-vitro* dissolution evaluation was determined to measure the rate and extent of dissolution or release of the drug substances from given nanosuspension in specified conditions. The % of drug release was calculated and plotted against time. The % drug release was determined from the VD3 nanosuspension prepared by using the polymer Eudragit RS100/RL100 with poloxamer 407. It was observed that the formulation shows the steady release of VD3 from nanosuspension.

Among all the formulations it was observed that the formulation VD3-F4 show the maximum drug release at the end of 10 hr it shows 99.87% of drug release.

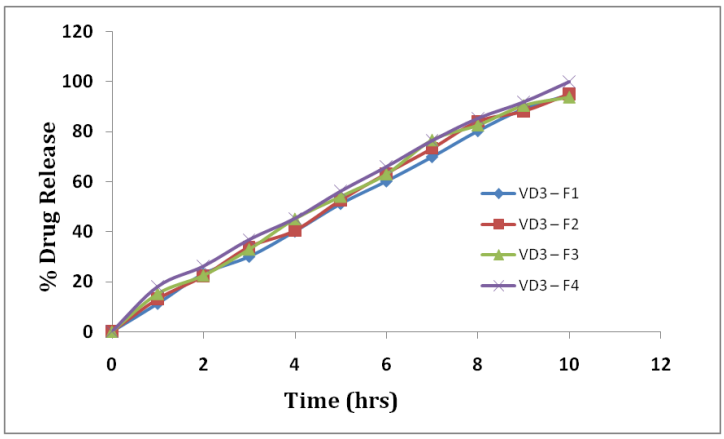

Figure 3: *In vitro* drug release from nanosuspension

It has been observed that the pure drug showed a poor dissolution profile; about 50 % of the drug was dissolved in 10 h because of poor aqueous solubility. After 10 h, almost 97 % VD3 was released from nanosuspension, while almost 78 % drug was released from the marketed formulation, which is almost 19 % less than nanosuspension (**Fig.4**).

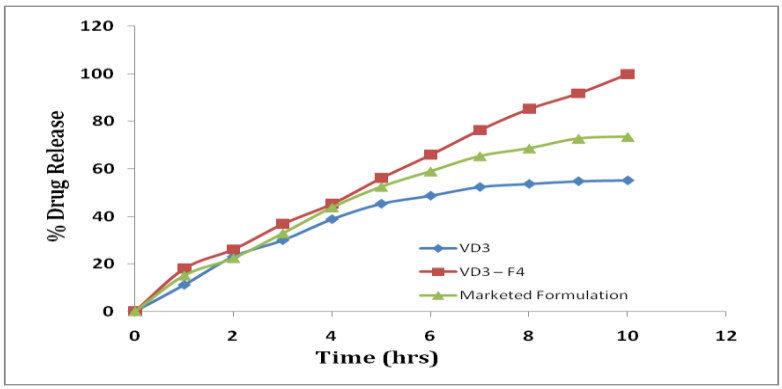

Figure 4: Comparison of % drug release profile with pure drug and marketed formulation

Steady release of the drug from nanosuspension, suggested a homogeneous and finer distribution of VD3 molecule in the polymer matrix, which enhance its dissolution profile. Properties of polymers also affectthe release of drugs. At pH ≥ 5.5 the the carboxylic acid group of ELD is transformed to the carboxylate group it induced pores on the polymer surface through which the drug diffuse into the dissolution medium.

CONCLUSION

In conclusion, the present demostrated the development of aqueous base VD3 nanosuspension by oil in water emulsion (o/w) process. The developed nanosuspensions were characterized by various analytical techniques such as FTIR, DSC and SEM. It has been found that in the nanosuspension the interaction between drug molecule (VD3) and the polymer is physical interaction. Hence it cannot affect the purity and efficacy of the drug molecules. In addition, the aqueous solubility of the VD3 has also been increased about 5 times in VD3 nanosuspension. Further, an in-vitro drug release study illustrate that VD3 released in steady manner from nanosuspension. As compared to marketed formulation 28% more drug have been release from nanosuspension which shows the success of nanosuspension formulation. This study could help to release aqueous based formulation of VD3.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

REFERENCES

1. V.K. Maurya and M.Aggarwal, *The Journal of Steroid Biochemistry and Molecular Biolog*, **190**, (2019).
2. Y.Luo, Z.Teng and Q.Wang, *Journal of Agricultural and Food Chemistry*, **60**, 3 (2012).
3. R.Vieth, 2005, The pharmacology of vitamin D, including fortification strategies in D.

Feldman and F. Glorieux (Eds.), Elsevier Academy Press, New York, pp. 995–1015.
4. D.L.Trump, K.K Deeb and C.S Johnson, *Cancer Journal*,**16**,1, (2010).
5. L.M.Schmitz, A. Kinner, K. Althoff, K. Rosenthal, S. Lütz, *ChemBioChem*, **22**,13, (2021).
6. M.Gonnet, L.Lethuaut and Frank Boury, *Journal of Controlled Release*, **146**, 3,(2010).
7. N.Ali, *Journal of Infection and Public Health*,**13**,10,(2020).
8. J.Feige, T.Moser, L.Bieler, K.Schwenker, L. Hauer andJ.Sellner, *Nutrients* ,**12**,3 (2020).
9. A. Drincic, L. Armas, E. Diest, R. P. Heaney, *Obesity*, **20**, 7, (2012).
10. N. Al-Bayyari, R. Hailat, H. Subih, H. Alkhalidy and A. Eaton , *British Journal of Nutrition* , **125** ,2,(2021).
11. P.Ravani, F.Malberti, G.Tripepi, P.Pecchini, S. Cutrupi, P. Pizzini, F. Mallamaci and C.Zoccali, *Kidney International*, **75**,1,(2009).
12. R.K. Marwaha, M.Verma, A.Walekar, R.Sonawane and C.Trivedi, *Journal of Orthopaedics*,**35**, (2023)
13. V.K.Maurya, K.Bashir and M.Aggarwal ,*The Journal of Steroid Biochemistry and Molecular Biology*,**196,** (2020).
14. R.Gupta, C.Behera, G.Paudwal, N.Rawat, A. Baldi and P.N. Gupt, *AAPS PharmSciTech*, **20**,11, (2019).