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Original Article

Delayed Release HPMC Capsules for Efficient Delivery of Cholecalciferol Solid Dispersion

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ABSTRACT

Introduction: The deficiency of Vitamin D is associated with an increased risk of various diseases and deficiency of this Vitamin is recognized in individuals all over the world. Therefore, the intake of Vitamin D has become essential. **Objectives:** Cholecalciferol (Vitamin D_3) is a poorly soluble molecule and it is very sensitive to degradation under environmental factors such as light, temperature, and oxygen. Its stability is also affected adversely under acidic conditions. Therefore, solid dispersion-based formulation for cholecalciferol was developed and encapsulated in delayed release hydroxypropylmethyl cellulose (HPMC) capsules. **Materials and Methods:** Cholecalciferol solid dispersion was developed and characterized by Fourier transform–infra red spectroscopy (FTIR), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and X-ray diffraction analysis. The effect of various concentrations of cholecalciferol formulations on the viability of Caco-2 cells was determined by using MTT assay. Dissolution profile and stability study of the developed product was also evaluated. **Results:** The results demonstrated improved solubility of cholecalciferol in solid dispersion-based formulation. The drug content of solid dispersions was in the order of 91±2.3% and various studies showed the amorphous form of cholecalciferol in the solid dispersion. The cell viability assay in Caco-2 cells demonstrated that the surfactant used in the solid dispersion formulation of cholecalciferol had no adverse effect on intestinal cells. Further, dissolution profile of HPMC capsule encapsulated solid dispersion showed improved dissolution of cholecalciferol. Moreover, the stability study indicated no significant changes in the cholecalciferol content in the developed formulation under storage at experimental conditions. **Conclusion:** The solid dispersion-based formulation of cholecalciferol exhibited improved solubility and found to be compatible with Caco-2 cells. The delayed release HPMC capsule encapsulated solid dispersion of cholecalciferol (DRHCap-SD) showed improved dissolution and acceptable stability profile and this represent a potential delivery system for oral administration of cholecalciferol.

Keywords: Solid dispersion, Cholecalciferol, HPMC capsule, Caco-2 cells, Oral delivery, Dissolution.

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INTRODUCTION

The deficiency of Vitamin D is gaining immense recognition as a serious health concern leading to a variety of health issues.[1,2] This vitamin is synthesized in the skin following the exposure of sunlight or this micronutrient can also be obtained from food sources.[3] Food based options for this Vitamin is scanty and deficiency of Vitamin D has been recognized in many countries.[4,5] Limited exposure to the sunlight and sedentary lifestyle is the main factors associated with the deficiency of Vitamin D. The deficiency of Vitamin D is linked with an increased susceptibility towards many ailments such as diabetes, cancers, cognitive decline and depression.[1,2,6,7] Vitamin D is an essential micronutrient owing to its ability to maintain calcium and phosphorus concentrations at the desired level by improving the ability of the intestine to absorb calcium and phosphorus from the food sources. The Vitamin D deficiency leads to rickets, osteomalacia, hyperparathyroidism, and osteoporosis.[8,9] Cardiovascular mortality has also been reported in type 2 diabetes mellitus patients with Vitamin D deficiency.[10] The regular dose of Vitamin D (about 2000 IU/d) was reported to reduce the risk of developing type 1 diabetes as its low levels are associated with insulinemia and glucose intolerance.[11] The potential of Vitamin D derivatives as an antitumor molecule has been documented owing to their property of hampering angiogenesis.[12] Keeping all these aspects into considerations, the intake of Vitamin D has become essential.

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Vitamin D is a hydrophobic micronutrient and it is a necessary component in the human diet for the good health of the individual. Two main chemical versions of Vitamin D include Vitamin D_2, called as ergocalciferol and D_3, called as cholecalciferol.[13] Cholecalciferol, which is generally formed in the skin following sunlight exposure, is more potent that ergocalciferol. Depending on the environmental condition, cholecalciferol may find different chemical versions including calciol, calcidiol, and calcitriol. The active form of cholecalciferol is calcitriol (chemically known as 1, 25-dihydroxyvitamin D_3), which is vital for calcium and phosphorus homeostasis, bone metabolism, blood pressure, reabsorption of calcium in the kidney, and secretion of insulin.[14]

The insufficient exposure to sunlight and disease conditions such as hyperparathyroidism, obesity and inflammatory bowel disease can lead to cholecalciferol deficiency.[15] The sources of cholecalciferol are very limited (e.g., dairy products, beef, liver, egg yolk, and fish),[16] leading to the great demand for enrichment of food and beverages with cholecalciferol. 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.[14] Moreover, it has been reported 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.[17] Keeping these aspects into consideration, novel formulation strategies are required for the efficient delivery of cholecalciferol.[18] Various delivery options have been explored to prevent the degradation of cholecalciferol including casein micelles,[19] zein nanoparticles coated with carboxymethyl chitosan,[14] carboxymethyl chitosan-soy protein complex nanoparticles,[20] solid dispersion,[21] and microspheres.[22]

In the present study a solid dispersion-based formulation was developed to improve the solubility of cholecalciferol and developed solid dispersion was subsequently encapsulated in the HPMC based delayed release capsules which offer protection of cholecalciferol from the low pH of the stomach. The HPMC capsules formulation (DRHCap-SD) delay the release of cholecalciferol until the capsule is in the intestine (i.e. pH>5.5). The solid dispersion-based formulation of cholecalciferol was characterized by FTIR, DSC, SEM, and X-ray diffraction analysis. Furthermore, as the developed solid dispersion comprising surfactant is intended for oral administration, therefore, the influence of formulation on the activity of Caco-2 cells was investigated. Subsequently, the dissolution profile of DRHCap-SD was evaluated in the biorelevant gastric and intestinal fluid, and thereafter stability profile of the formulation at various storage conditions was assessed.

MATERIALS AND METHODS

Materials

Cholecalciferol, Trypsin-EDTA, MTT dye and HBSS were obtained from Sigma Chemical Co., USA. Polyvinyl-pyrrolidone PVP K-30 was purchased from SD Fine-Chem Limited Mumbai (India). Dulbecco's Phosphate Buffered Saline and EMEM were purchased from Hi Media Labs Pvt. Ltd., Mumbai (India). Delayed release HPMC capsules were obtained from ACG Capsules, Mumbai, India. FBS and Penicillin-streptomycin were obtained Invitrogen Corporation and Thermo-scientific, respectively. Caco-2 cells were obtained from NCCS, Pune.

Preparation of DRHCap-SD of Cholecalciferol

The solid dispersion of cholecalciferol in PVP-K30 comprising various weight ratios (1:200, 1:400, 1:600, 1:800 of CCF: PVP K-30, designated as SD1, SD2, SD3 and SD4, respectively) were prepared by solvent evaporation method. Required quantity of cholecalciferol and PVP K-30 was separately dissolved in the minimum amount of ethanol. The solution containing cholecalciferol was poured in solution containing PVP K-30 and the system was mixed using magnetic stirrer. After 20 min of mixing, poloxamer (PVP: poloxamer = 10:1) was added slowly to the solution mixture and solvent was evaporated and prepared formulation was placed under desiccators for 48 hr to dry completely. After drying the hardened mixtures was pulverized with porcelain pestle and sieved through mesh #18. The solid dispersion was filled in delayed release HPMC capsule and subsequently the product (DRHCap-SD) was placed in air tight container.

Physical Mixture Preparation

The preparation of physical mixture of cholecalciferol with carrier polymers was carried out in same proportion as in solid dispersion with porcelain mortar pestle to ensure uniform product and sieved through mesh #18 and stored in air tight container.

Percentage Practical Yield

Percentage practical yield was calculated to investigate efficiency of method. Prepared solid dispersions-based formulation was collected and weighed to estimate practical yield by employing following equation.

$$\% \text{ Practical yield} = \frac{\text{practical mass (solid dispersion)}}{\textit{theoretical mass}} \times 100$$

Drug Content

Drug content was estimated by dissolving 100mg of formulation in 1ml of mobile phase (acetonitrile: water = 99:1) and the solution was vortexed for 20 min and then centrifuged, filtered and concentration of cholecalciferol was determined at 265 nm using a HPLC method.[23] Separation was achieved by using

an Chromolith® Performance RP-18 endcapped 100-4.6mm HPLC column. The mobile phase flow rate was 1ml/min and all chromatographic separations were performed at ambient temperature. The drug content was obtained by using following equation.

$$\% \text{ drug content} = \frac{\textit{actual amount of CCF content present in the solid dispersion}}{\textit{theoretical amount of CCF in solid dispersion}} \times 100$$

Solubility Determination

Excess amount of cholecalciferol and its solid dispersion were dissolved in 1 ml of PBS buffer 7.4 with continuous vertexing and incubated for 24 hr in water bath (37±0.5°C). Thereafter, samples were subjected to centrifugation at 10,000g for 10 min and supernatant were analyzed using HPLC at 265 nm.

FT-IR Spectroscopy

FT-IR spectra of cholecalciferol, polymers and solid dispersion were obtained using FT-IR (Perkin Elmer Spectrum, Two FT-IR Spectrometer version 10.03.06) using KBr pellet method. Briefly, 5mg of samples were uniformly triturated with 100 mg KBr and KBr discs were obtained using concentional method. The instrument performed under dry air purge at 4000-400 cm^{-1} scanning range.

Scanning Electron Microscopy (SEM)

Surface morphology of cholecalciferol, PVP-K30 and solid dispersion was examined by using SEM (JEOL, Japan). Formulations and excipients were separately fixed on a brass stub and were sputter coated (JEOL JC 3000FC Autofine coater) with platinum-palladium alloy layer (approx. 3-5nm) for 150 sec at 30W, to made it electrically conductive, and images are obtained through secondary electron detector at accelerating voltage ranging from 10KV-15 KV.

Differential Scanning Calorimetry Analysis

Thermal analysis of cholecalciferol and its solid dispersion formulation was performed using differential scanning calorimetry. Weighed quantity of samples were kept in aluminum pans and heating was done under nitrogen flow (30ml\min) with a scanning rate of 10°C min^{-1} from 20 to 220°C.

X-Ray Diffraction Analysis

XRD analysis of powder samples was conducted using a diffractometer. The diffraction patterns were recorded using a Bruker D8 venture diffractometer system (XPERT-PRO) with INCOATEC microfocus X-ray source (Mo Kα) at 45 kV and 40 mA. Various samples were scanned with scan step time 10.16 sec from 5° to 80° (diffraction angle 2θ) at measurement temperature of 25°C.

Effect of Formulation of Viability of Caco-2 Cells

In this study, the solid dispersion of cholecalciferol comprises surfactant in addition to other excipients and the formulation is intended for oral administration. Therefore, influence of formulation on the activity of the Caco-2 cells was determined. Caco-2 cells are widely explored as a model system for screening of intestinal absorption and cytotoxicity.[24] The effect of the formulations on the activity of Caco-2 cells was assessed by MTT assay. Caco-2 cells were grown on 75cm^2 plastic culture flasks (NUNC) in EMEM with Earle's salts, L-Glutamine and non-essential amino acids containing 2.38 g/l HEPES, 2 g/l sodium bicarbonate, 10% (v/v) FBS, 100 IU/mL penicillin and 100 µg/ml streptomycin. Caco-2 cell lines were incubated at 37°C inside the incubator with 5% CO_2 and 95% relative humidity. Caco-2 cells were used between passages 40 and 45 in the experiment. The cells were routinely maintained and trypsinized in T75 tissue culture flasks.

In order to estimate the effect of the formulation on viability of Caco-2 cells, they were seeded at a density of $2x10^4$ cells/well inside the humidified CO_2 incubator for 96 hr and the media was changed at an alternate day. Before the treatment, the exhausted media was removed. Once the cells formed a monolayer in a 96 well plates, different concentrations of formulations in HBSS solution were added to each well and kept for 2 hr incubation. Thereafter, 20 µl of MTT solution (2.5 mg/ml) prepared in cold PBS was added in each well and the cells were again incubated for another 4 hr to form formazan crystals. The formazan crystals were dissolved by adding 150 µl of DMSO using an orbital shaker and the absorbance was recorded at 570 nm and percent viability was calculated.

Dissolution Study

The *in vitro* dissolution study of the solid dispersion encapsulated in delayed release HPMC capsule was conducted using two-stage dissolution[25] using a dissolution apparatus (Lab India DS 8000) and dissolution profile was compared with native solid dispersion, physical mixture and cholecalciferol. The biorelevant media (simulated gastric fluid and simulated intestinal fluid) were prepared as reported previously.[26,27] The experiment was performed using a paddle apparatus operated at 100 rpm and the dissolution media was kept at 37±0.5°C. In the first stage, dissolution was conducted in simulated gastric fluid (SGF) medium (250 ml) and subsequently double strength simulated intestinal fluid (SIF) media (250ml) was added for the second stage of experiment. Dissolution profile was estimated by removing 2 ml samples at different time intervals, followed by filtration through 0.45 µm PVDF filter and an equal volume of dissolution medium was replaced. The collected samples were estimated for cholecalciferol by HPLC at 265 nm.

Table 1: Characterization of solid dispersion formulation.

SD formulation	Solubility (µg/ml)	Drug content (%)	Yield (%)
SD1 (1:200 w/w)	259±11	83.4±4.2	82.7±3.9
SD2 (1:400 w/w)	302±10	86.1±3.6	85.2±4.1
SD3 (1:600 w/w)	658±24	91.7±2.3	88.6±4.3
SD4 (1:800 w/w)	387±14	87.8±2.9	85.5±3.8

Flow Properties

Cholecalciferol SDs and its physical mixture were evaluated for flow properties. Angle of repose was evaluated by passing the samples to flow through funnels and the angle between horizontal surface and slope of the heap of samples was noted. Bulk density and tapped density (after 50 times tapping) were determined using conventional method and Carr's compressibility Index (CI), angle of repose (Θ) and Hausner's Ratio were calculated according to following formulas:

$$\Theta = \tan^{-1}\frac{h}{r}$$

Where, h and r are height and radius of the powder pile, respectively.

$$\text{Compressibility index} = \frac{(tapped\ density - bulk\ density)}{tapped\ density} \times 100$$

$$\text{Hausner's ratio} = \frac{tapped\ density}{bulk\ density}$$

Stability Study

The DRHCap-SD of cholecalciferol and solid dispersion formulations were placed in sealed containers and stability study was performed by storing the samples at 4°C (refrigerator), 25°C (60%RH), and 30°C (65%RH) for three months. Formulations were removed from stability chamber after every month and analyzed for drug content.

Statistical Analysis

The data are shown as mean ± S.D. The statistical analysis of data was performed by using ANOVA and Student's *t*-test. The value of *p* less than 0.05 was designated as significant.

RESULTS

Preparation of DRHCap-SD of Cholecalciferol

The solid dispersion in present study was developed by solvent evaporation technique. The percent yield of formulation was in range of 82-88% and not much variation was observed in different batches (Table 1). The drug content of the formulation was in range of 83-91% reflecting the suitability of the solid dispersion preparation method with regard to content uniformity. The formulation SD3 was found to be good and exhibited the percent practical yield and drug content as 88.6±4.3% and 91.7±2.3%, respectively. The solubility profile of various solid dispersion formulations (Table 1) showed significantly improved solubility of the cholecalciferol in formulations compared to the native cholecalciferol, which exhibited solubility in PBS buffer (pH 7.4) as 1.6±0.11 µg/ml. The solubility of cholecalciferol was greatly improved in the solid dispersion (SD3). However, further enhancement of PVP K30 in the solid dispersion (SD4) leads to supersaturation and cause no further enhancement in solubility of the cholecalciferol.

FT-IR Spectroscopy

FTIR spectroscopy was conducted to investigate any interactions between the cholecalciferol and excipients (Figure 1). Cholecalciferol spectrum shows broad absorption band at 3411cm^{-1} due to stretching vibration of hydroxyl group and sharp band at 2934 cm^{-1} and 2890 cm^{-1} due to the symmetric and asymmetric vibration of C-H bonds, respectively. The stretching vibration of C=C and C=O bonds were seen at 1637 cm^{-1} and 1458 cm^{-1}, respectively.

Scanning Electron Microscopy

The surface characteristics of the developed solid dispersions was examined by scanning electron microscopy (Figure 2). The SEM photomicrograph showed the crystalline nature of cholecalciferol

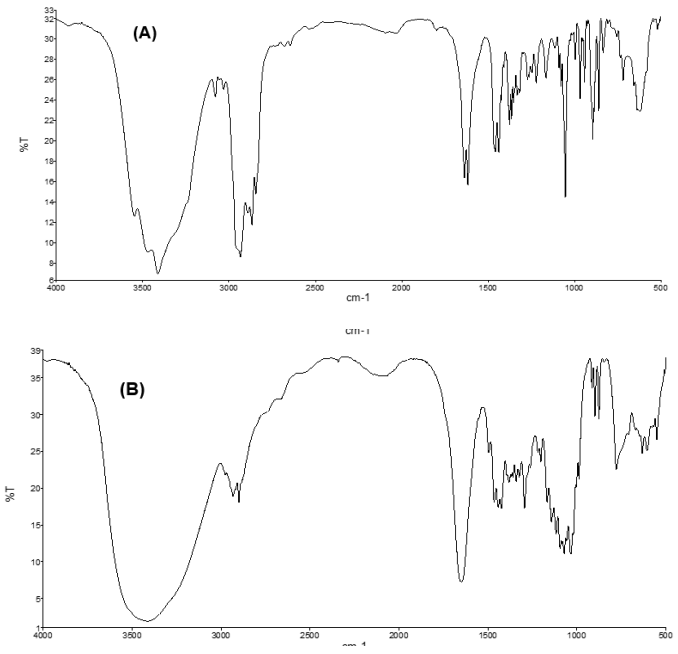

Figure 1: IR spectroscopy of cholecalciferol (A) and solid dispersion formulation (B) at scanning range of 4000-400 cm^{-1}. Characteristics functional group cholecalciferol is retained in the solid dispersion formulation.

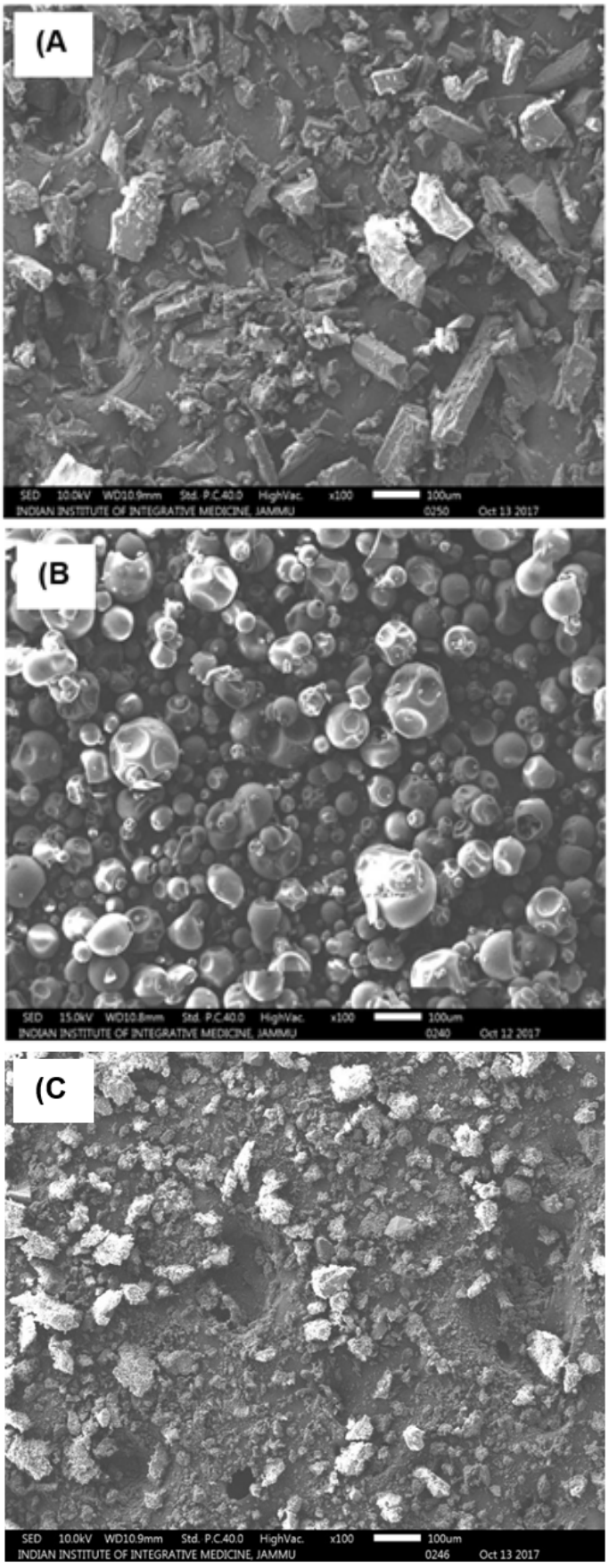

Figure 2: Scanning electron microscopic images of (A) Cholecalciferol, (B) PVP-K30, (C) CCF-SD-PVP at accelerating voltage ranging from 10KV-15 KV.

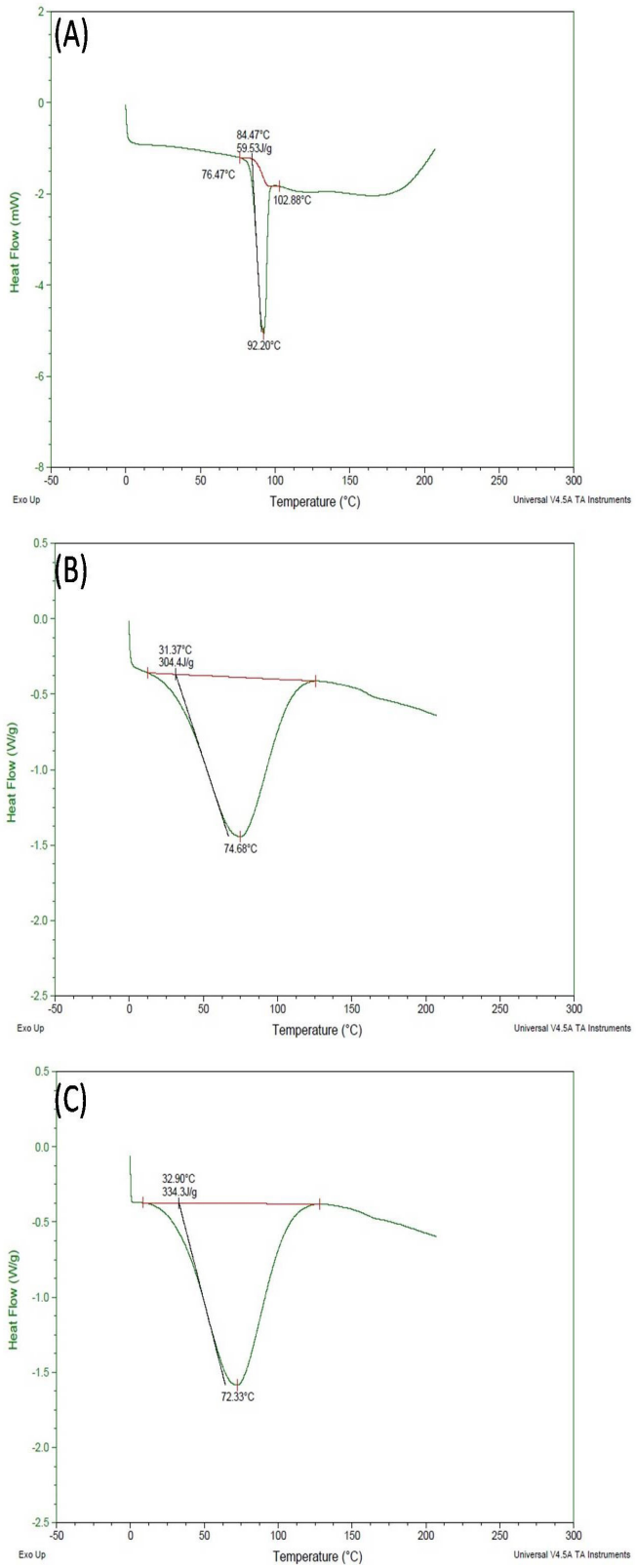

Figure 3: Differential scanning calorimetry analysis of (A) Cholecalciferol and (B) PVP-K30 and (C) Solid dispersion formulation (CCF-SD-PVP) at a scanning rate of 10°C min^{-1} from 20 to 220°C.

with irregular orthorhombic crystals (Figure 2A.), while PVP K-30 showed amorphous nature with spherical shaped particles (Figure 2B). The developed solid dispersions formulation (Figure 2C) exhibited no crystalline feature of cholecalciferol and formulation appeared as small aggregates of amorphous particles.

Differential Scanning Calorimetry (DSC) Analysis

The DSC study was conducted for the characterization of nature of cholecalciferol within the solid dispersion formulation. The morphological investigation of formulation using the SEM has suggested the transitions of cholecalciferol from crystalline to amorphous phase in the process of obtaining solid dispersions and the DSC study further confirm the amorphous form of the cholecalciferol in the formulation. DSC analysis provides important information about the melting profile of a crystalline substances. The thermal behavior of cholecalciferol can be adjudged by a sharp endothermic peak corresponding to the temperature 92.20°C (Figure 3A) characteristics of its crystalline structure, which is in accordance to the scanning electron microscopic examination. The PVP K-30 exhibited broad endothermic curve at 74.68 ranging from 31.37°C to 135°C indicating the loss of water (Figure 3B). Solid dispersion obtained by solvent evaporation technique did not show any endothermic peak event corresponding to melting of cholecalciferol at 92.20°C (Figure 3C), indicating the formation of solid dispersion where the drug converted from crystalline to amorphous form.

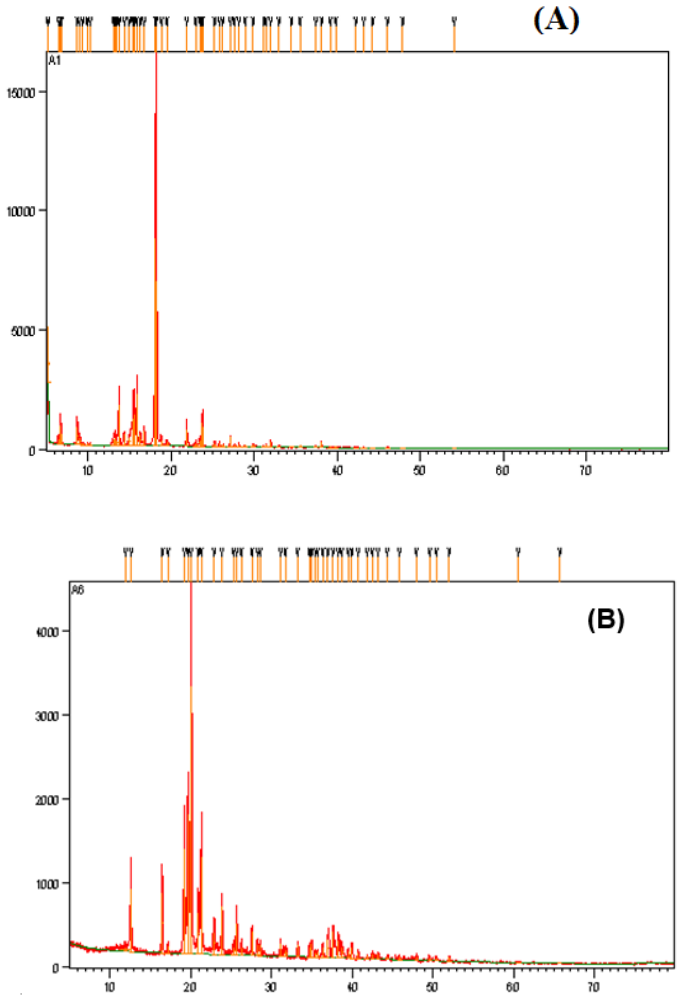

Figure 4: X-ray diffraction analysis of (A) Cholecalciferol and (B) Solid dispersion formulation (CCF-SD-PVP) at scanning range from 5° to 80° (diffraction angle 2θ).

X-Ray Diffraction Analysis

Cholecalciferol and its solid dispersion formulation (CCF-SD-PVP) was characterized using X-ray diffraction analysis at diffraction angle 2θ (Figure 4). Cholecalciferol exhibited crystalline peaks at 2θ = 5.18°, 13.69°, 15.87°, 18.15° and 18.81° due to its crystalline characteristics. This observation is in accordance with a previous study.[28] On contrary, the characteristics peaks of cholecalciferol were not appeared in the solid dispersion formulation.

Effect of Formulation on Viability of Caco-2 Cells

In our study, the effect of various concentrations of cholecalciferol formulations (CCF-SD-PVP) on the activity of Caco-2 cells was evaluated by MTT assay (Figure 5). The results are indicating that upon the treatment equivalent to 10, 20, 50 and 100 µg/ml of cholecalciferol in CCF-SD-PVP, the viability of the caco-2 cells was more than 80%. It has been demonstrated in the study that the surfactant used in the solid dispersion formulation is devoid of any cytotoxic effect on Caco-2 cells and the observations are in accordance to a previous study.[29]

In vitro Dissolution

Cholecalciferol is an important micronutrient and it has implication in various cellular events required for normal

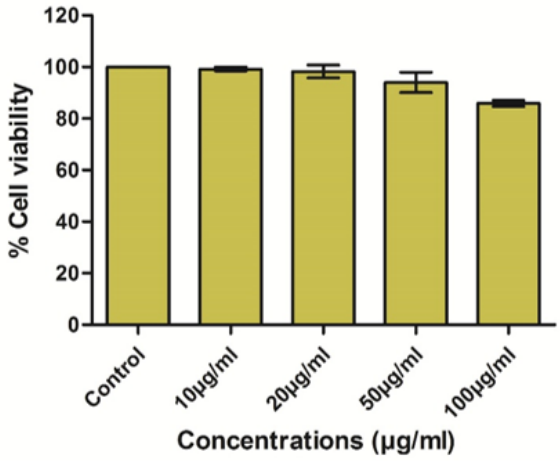

Figure 5: Evaluation of compatibility of the formulation with Caco-2 cells. Various concentration of the cholecalciferol solid dispersion-based formulations was incubated with Caco-2 cells and the viability of Caco-2 cells was assessed by using MTT assay. The solid dispersion formulation is devoid of any cytotoxic effect on Caco-2 cells.

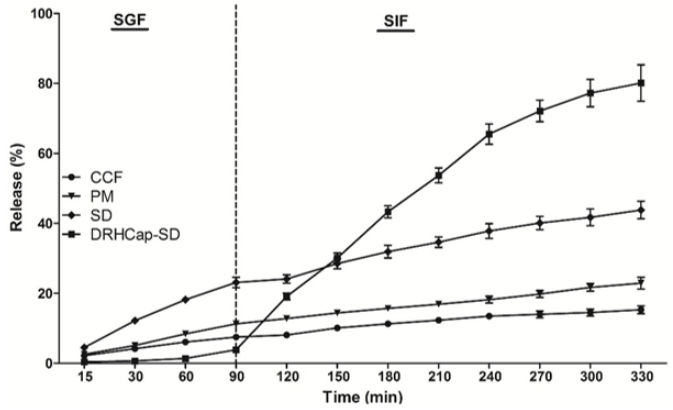

Figure 6: Dissolution profile of cholecalciferol (CCF), physical mixture (PM), solid dispersion (SD), and solid dispersion in delayed release HPMC capsule (DRHCap-SD) in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Dissolution study was conducted for 90 min in SGF and 240 min in SIF using dissolution apparatus with paddle operated at 100 rpm and the temperature of dissolution media was kept at 37±0.5°C.

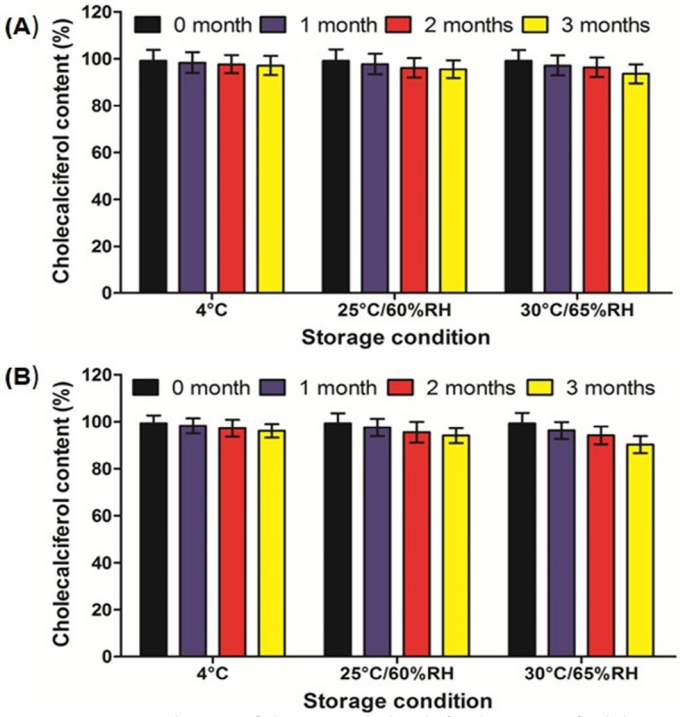

Figure 7: Evaluation of change in cholecalciferol content of solid dispersion-based formulation after three months of storage at different conditions (4°C, 25°C/60%RH and 30°C/65%RH): (A) DRHCap-SD (B) SD.

Table 2: Evaluation of flow properties of solid dispersion and physical mixture.

Bulk property	Solid dispersion	Physical mixture
Angle of repose (°)	32.1±0.9	35±1.1
Bulk density (g/ml)	615±14	601±12
Tapped density (g/ml)	722±15	741±16
Compressibility index (%)	14.81±0.3	18.89±0.4
Hausner's ratio	1.17±0.01	1.23±0.01

functioning of the body. This lipophilic agent is very sensitive to the environmental conditions[14] and its degradation rate is more at low pH.[18] Keeping these aspects into consideration in the present study, cholecalciferol solid dispersion was developed for the solubility improvement and developed solid dispersion was encapsulated in relayed release HPMC capsules to limit the exposure of cholecalciferol to acidic condition. In this study, a two-stage dissolution in simulated body fluids was performed. The first stage mimics the condition in gastric fluid and utilizes SGF (pH 1.8). The second stage mimic the cholecalciferol release in intestinal micro-environment and utilizes SIF (pH 6.8). Cholecalciferol is lipophilic micronutrient and showed poor dissolution in simulated body fluids. In SGF, CCF-PVP-SD (SD) formulation exhibited improved dissolution (23%) compared to the CCF, CCF-PM, and DRHCap-SD. In DRHCap-SD formulation, HPMC capsule delays the release of cholecalciferol in SGF therefore DRHCap-SD showed poor dissolution profile in SGF (Figure 6). However, in SIF, DRHCap-SD causes marked improvement in the dissolution of cholecalciferol (~ 80%) in comparison to the CCF, CCF-PM, and CCF-SD-PVP (SD).

Flow Properties

The bulk properties of the developed solid dispersion and their physical mixture were determined and compared with the USP specifications.[30] The angle of repose of CCF-SD-PVP and its physical mixture was good (Table 2). However solid dispersion-based formulation had an edge over its physical mixture for this parameter. The compressibility index of the CCF-SD-PVP was found good, whereas for physical mixture the value of the compressibility index was found in fair category. Similarly, the Hausner's ratio of the CCF-SD-PVP was good, whereas for physical mixture the value of this ratio was found as fair.

Stability Study

The stability of the cholecalciferol is influenced by the environmental factors such as light, oxygen, and temperature. Thus, the stability of the DRHCap-SD and SD was evaluated by storing these formulations at different storage conditions of temperature and humidity. The formulations DRHCap-SD and SD were stored at 4°C, 25°C/60%RH, and 30°C/65%RH for a period of three months and after every month samples were taken for analysis of CCF content. The results showed that there was a decrease in cholecalciferol concentration in DRHCap-SD and SD following storing them at 30°C/65%RH for three months. However, there was no remarkable difference in the cholecalciferol content in DRHCap-SD and SD formulation after three months of storage at 4°C and 25°C/60%RH (Figure 7). These findings are in accordance to a previous report which demonstrated improved the stability of calcitriol with a lipid-based formulation.[21] Also, a polymeric formulation demonstrated good stability profile of cholecalciferol at different conditions of storage.[31]

DISCUSSION

Cholecalciferol is an important micronutrient having significant impact on human health. The physicochemical characteristics and in-tum the bioactivity of cholecalciferol is influenced by the environmental conditions and the microenvironment of the gut. Therefore, in order to realize full therapeutic benefit of cholecalciferol rationally designed formulation strategies are required. In this direction, a solid dispersion-based formulation was developed to improve the solubility of cholecalciferol and subsequently developed solid dispersion was encapsulated in delayed release HPMC capsules which offer protection of cholecalciferol from the low pH of the stomach. This dual-purpose formulation holds the potential for efficient oral delivery of this micronutrient. Delayed release HPMC capsules prevent degradation of cholecalciferol by avoiding exposure to stomach acids. These capsules delay the release of formulation by upto 60 min or until the capsule is in the intestine (i.e. pH>5.5). Further, these capsules are easier and more convenient way to develop modified release product as they eliminate the need for the additional protective coating of formulation or capsule. The delayed release HPMC capsules are ideal choice for acid sensitive product like cholecalciferol.

The enhancement in solubility of cholecalciferol from developed solid dispersion formulation may be due to various reasons including surfactant aided solubilization and improved wetting property, conversion into amorphous form and size reduction. The analysis of spectra of solid dispersion formulation showed that the characteristics functional group cholecalciferol is retained in the solid dispersion formulation. Hence the chemical structure of cholecalciferol is likely to be unaffected during solid dispersion formulation development. The changes in the morphology of formulation suggested transitions from crystalline to amorphous phase in the process of obtaining solid dispersions and the DSC study further confirm the amorphous form of the cholecalciferol in the formulation. The XRD study showed that the characteristics peaks of cholecalciferol were not present in the solid dispersion formulation. The reason could be the change in the crystalline characteristics of cholecalciferol to amorphous nature, which is supported by an earlier report.[32] The amorphous form of the molecule is normally associated with the feature of higher solubility as compared to the crystalline form.[33]

Solid dispersion of cholecalciferol in this study comprises polymer and surfactant. Surfactants are widely employed in pharmaceutical compositions of a poorly water-soluble drug and they improve solubility of a drug via micellar solubilization and also possess potential for modulation of membrane permeability.[34] However, surfactants are notorious for their local irritation effect, membrane disruption, and cellular death. Therefore, surfactant containing compositions should be assessed for cellular toxicity[35] and Caco-2 cells are commonly used for this objective. Surfactants cause modulation in Caco-2 permeability and there is correlation between paracellular transport with viability.[36,37] We have investigated the effect of formulation on viability of Caco-2 cells and the results showed that the surfactant used in the solid dispersion formulation is devoid of any cytotoxic effect on Caco-2 cells which is in accordance to a previous report.[29]

The improvement in the dissolution profile of cholecalciferol from DRHCap-SD can be attributed to the protection of the cholecalciferol in acidic SGF condition and this can also be associated with the reduction in particle size of the cholecalciferol and its possible amorphization of solid dispersion, enhanced wetting of the drug and the possible solubilization effect of the polymer or surfactant. The bulk properties of the developed solid dispersion and their physical mixture were evaluated and the results demonstrated that the flow properties were improved by formulation of solid dispersion. Cholecalciferol is a sensitive micronutrient whose stability is affected by environmental factors. The developed solid dispersion-based formulations could lead to immobilization of the cholecalciferol in polymer carriers leading to decreases in cholecalciferol mobility. In addition, improved stability may be attributed to the physical barrier for the penetration of oxidizing agents, whose exposure to cholecalciferol is hindered in the polymeric matrix-based solid dispersion formulation. Also, encapsulation in delayed release HPMC capsule further contribute to the improved stability of cholecalciferol in DRHCap-SD.

CONCLUSION

In this study, solid dispersion of cholecalciferol was successfully developed which were encapsulated in delayed release HPMC capsule. The solid dispersion of cholecalciferol was characterized by FTIR, SEM, DSC and X-ray diffraction analysis, and these techniques indicated the successful formation of the solid dispersion of cholecalciferol. The solid dispersion of cholecalciferol in this study consists of a surfactant, therefore, its effect on the activity of Caco-2 cells was evaluated in order to assess the safety of the formulation. The results showed that the surfactant used in the solid dispersion formulation exhibited no cytotoxic effect on Caco-2 cells. The dissolution study of DRHCap-SD and SD formulation of cholecalciferol was conducted in SGF and SIF medium. The study demonstrated improved dissolution of cholecalciferol from DRHCap-SD formulation. The storage stability study demonstrated no significant difference in the cholecalciferol content in the DRHCap-SD and SD formulations after storage at 4°C and 25°C/60%RH. The study demonstrated potential of DRHCap-SD formulation of cholecalciferol for further translational studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FTIR: Fourier transform–infra red spectroscopy; **DSC:** Differential scanning calorimetry; **SEM:** Scanning electron microscopy (SEM); **HPMC:** Hydroxypropylmethyl cellulose; **DRHCap-SD:** Delayed release HPMC capsule encapsulated solid dispersion; **CCF:** Cholecalciferol; **SGF:** Simulated gastric fluid; **SIF:** Simulated intestinal fluid.

SUMMARY

Solid dispersion-based formulation for cholecalciferol was developed to improve its solubility and encapsulated in delayed release HPMC capsules for protection of cholecalciferol from the acidic condition of the stomach. Cholecalciferol solid dispersion was developed and characterized by FTIR, DSC, SEM, and X-ray diffraction analysis. The results demonstrated improved solubility of cholecalciferol in solid dispersion-based formulation and various studies showed the amorphous form of cholecalciferol in the solid dispersion. The cell viability assay in Caco-2 cells demonstrated that the surfactant used in the solid dispersion formulation of cholecalciferol had no adverse effect on intestinal cells. Further, dissolution profile of HPMC capsule encapsulated solid dispersion showed improved dissolution of cholecalciferol. Moreover, the stability study indicated no significant changes in the cholecalciferol content in the developed formulation under storage at experimental conditions. The delayed release HPMC capsule encapsulated solid dispersion of cholecalciferol (DRHCap-SD) showed improved dissolution and acceptable stability profile and this represent a promising carrier system for oral delivery of cholecalciferol.

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