Chapter 6: Results and Discussion

6.1 Preformulation Studies of Cholecalciferol

6.1.1 Determination of solubility

The solubility of cholecalciferol in phosphate buffer saline (PBS, pH 7.4) was estimated using shake flask method (Konsoula and Jung, 2008). The solubility of cholecalciferol in phosphate buffer saline (PBS, pH 7.4) was found to be 1.8±0.3 µg/mL.

6.1.2 Determination of partition co-efficient

The partition behavior of cholecalciferol was examined in n-octanol: water and n-octanol: PBS (pH 6.8) system and the results are presented in Table 6.1. The results indicated the lipophilic nature of the cholecalciferol.

Table 6.1: Partition coefficient of cholecalciferol in n-octanol and PBS/water

Solvent system	Partition coefficient
n-Octanol : PBS (pH 6.8)	7.5±1.2
n-Octanol : water	8.4±0.2

6.1.3 Determination of melting point

The melting point of cholecalciferol was determined by melting point apparatus and it was found to be 83-85 °C.

6.2 Methods for Determination of Cholecalciferol

6.2.1 Standard curve of cholecalciferol in PBS

The stock solution of cholecalciferol was prepared in ethanol and subsequently different known concentration of solution were prepared using PBS 7.4. The absorbance of the solution of known concentration was then recorded at 265 nm using UV plate reader. The standard curve was obtained by plotting absorbance vs concentration (Fig. 6.1).

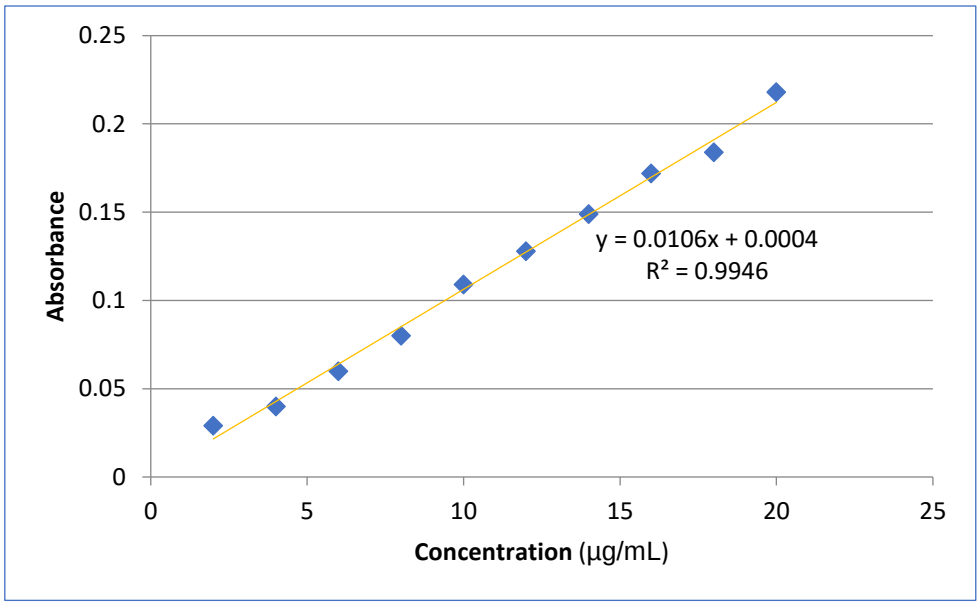

Figure 6.1: Standard curve of cholecalciferol in PBS (pH 7.4) at 265 nm.

6.2.2 Standard curve of cholecalciferol in ethanol

The stock solution of cholecalciferol was prepared in ethanol and subsequently different known concentration of solution were prepared using ethanol. The absorbance of the solution of the known concentration was then recorded at 265 nm using UV plate reader. The standard curve was obtained by plotting absorbance vs concentration (Fig. 6.2).

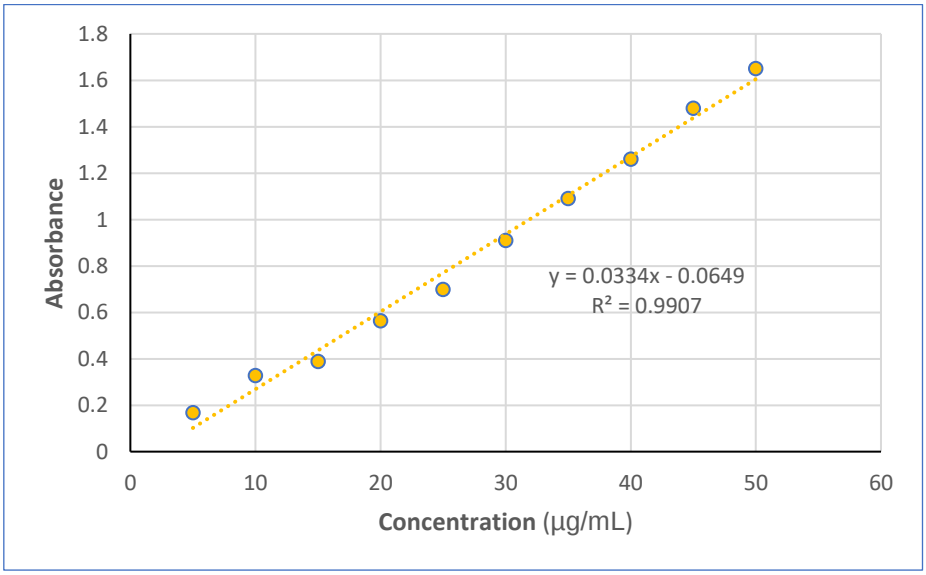

Figure 6.2: Standard curve of cholecalciferol in ethanol at 265 nm.

6.2.3. HPLC method for determination of cholecalciferol

The concentration of cholecalciferol was determined by a previously reported method (Temova and Roskar, 2016) using a HPLC system (Shimadzu UFLC, Shimadzu corporation, Japan). The methodology involved mobile phase flow rate of 1mL/min and wavelength of detection at 265 nm. In this method the chromatographic separations were carried out at the temperature of 40˚C. The HPLC chromatogram of the cholecalciferol is shown in Fig. 6.3, which shown a retention value of cholecalciferol at 10.566 min.

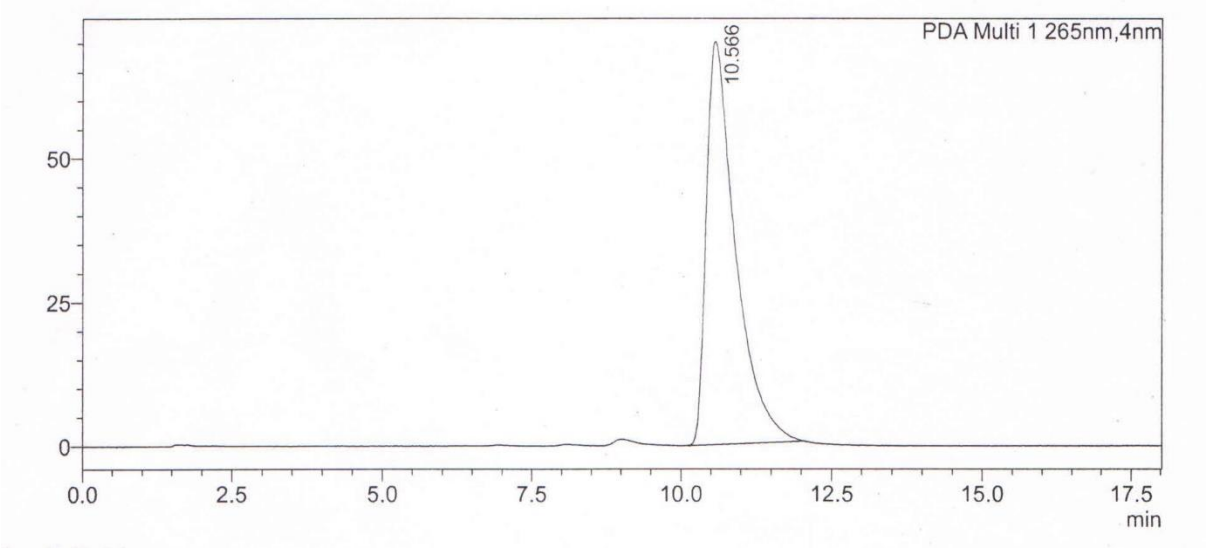

Figure 6.3: HPLC chromatogram of cholecalciferol in mobile phase comprising acetonitrile and water (99:1 v/v) at 265 nm.

6.3 Strategy I: Development and Characterization of Enteric Polymer-based Solid Dispersion for Cholecalciferol Delivery

6.3.1 Preparation of HPMCAS-based solid dispersion

Keeping in consideration the physicochemical characteristics of cholecalciferol, HPMCAS-based solid dispersion was attempted in this study for site-specific delivery. Solid dispersion is a potential delivery option to have good physical stability, improved dissolution, and oral bioavailability of poorly soluble drugs. One of the commercial

formulations of cholecalciferol (Calcirol, manufactured by Cadila Pharmaceuticals Limited) comprises 1.5 mg of cholecalciferol in 1-gram granules in sachet dosage form. With this background, we developed solid dispersion formulation comprising 1.5 mg of cholecalciferol and other excipients (PVP: 450 mg; HPMCAS: 450 mg; Poloxamer: 90 mg) with the total amount approximately equivalent to the commercial formulations. In one of our studies, it was found that 1:600 (w/w) ratio of cholecalciferol to solubilizing agent results in good solubility of cholecalciferol. PVP was used as solubilizing agent in developed formulation. HPMCAs was used as solubilizing agent and as an enteric polymer to prevent exposure of cholecalciferol from the acidic environment of the stomach. The effectiveness of HPMCAs as solid dispersion carrier has attracted the great attention. HPMCAs is an enteric polymer of particular importance as it is capable of initiating and maintaining supersaturation of compounds of diverse chemical structures and physical properties. The additional advantage of HPMCAs is that it acts as a precipitation inhibitor via generation of colloidal moieties in aqueous phase. Therefore, we used 1:600 ratio [Cholecalciferol: (PVP+HPMCAs) = 1.5 mg:900 mg = 1:600] in our formulation. Additionally, poloxamer was included in this composition to serve as surfactant which could be of advantage for hydrophobic molecule (cholecalciferol) and its amount was 90 mg to make the total amount approximately equivalent to the commercial formulations. In this study, solid dispersion was developed by solvent evaporation method. Percentage yield of HPMCAS-based cholecalciferol solid dispersion (CCF-SD-HPMCAS) and PVP based solid dispersion was (CCF-SD-PVP) was found to be 84.1% and 85.5%, respectively. The drug content of the enteric solid dispersions was in the order of 90% with a high degree of content uniformity.

6.3.2 Fourier transform – Infra red (FT-IR) spectroscopy

FTIR spectroscopy relies on the measurement of absorption of light by the vibration modes of particular chemical bonds in the molecule. The analysis of the vibration spectra of cholecalciferol, various excipients, physical mixtures, and solid dispersions indicates an interaction, if any, between cholecalciferol and polymeric material, because the vibration of atoms involved in this interaction can have altered frequency and intensity. The FTIR spectroscopy was carried out to ascertain any interaction between the cholecalciferol and the excipients (Fig. 6.4 – Fig. 6.11). Cholecalciferol spectrum shows broad absorption band at 3306 cm^{-1} attributed to stretching vibration of hydroxyl group. The spectra also showed band at 2940 cm^{-1} and 2868 cm^{-1} attributed to the symmetric and asymmetric vibration of C-H bonds, respectively. Additionally, the bands observed at 1635 cm^{-1} and 1459 cm^{-1} are owing to stretching vibration of C=C and C=O bonds (Fig. 6.4). The IR spectroscopy of PVP K-30 indicates that it acts as a proton acceptor owing to the carbonyl oxygen atoms or the nitrogen atom in the pyrrole moiety (the specific band was appeared approximately at 1637 cm^{-1}) (Fig. 6.6). Due to the steric hindrance phenomena, there was impediment for nitrogen atom for taking part in the molecular interactions, hence the carbonyl group is more prone to hydrogen bonding. FTIR spectra of poloxamer showed a wide peak at 3415 cm^{-1} for O-H functional group and C-H stretching peak was observed at 2955 cm^{-1}(Fig. 6.5). HPMCAS exhibited carbonyl stretching at 1740 cm^{-1} (Fig. 6.7). The IR spectroscopy showed that the characteristics functional moiety of the solid dispersion formulation had almost similar features as that of native cholecalciferol and polymer physical mixture. This experiment indicates absence of any molecular interactions, ruling out the possibility of alteration in the structural features of the active molecule.

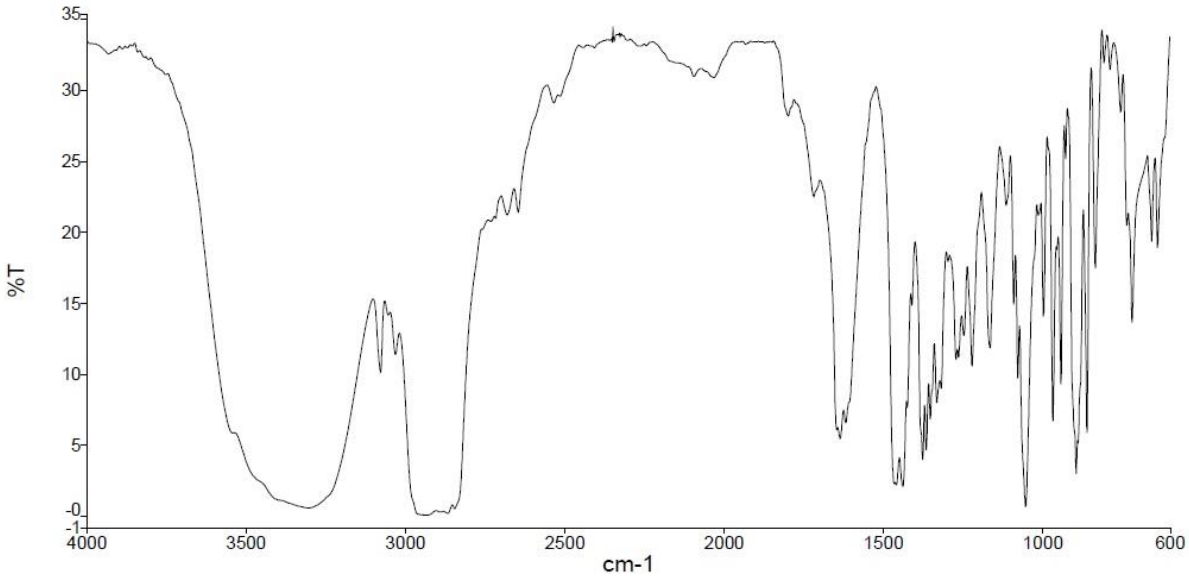

Figure 6.4: IR spectroscopy of Cholecalciferol at scanning range was of 4000-550 cm^{-1} using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.

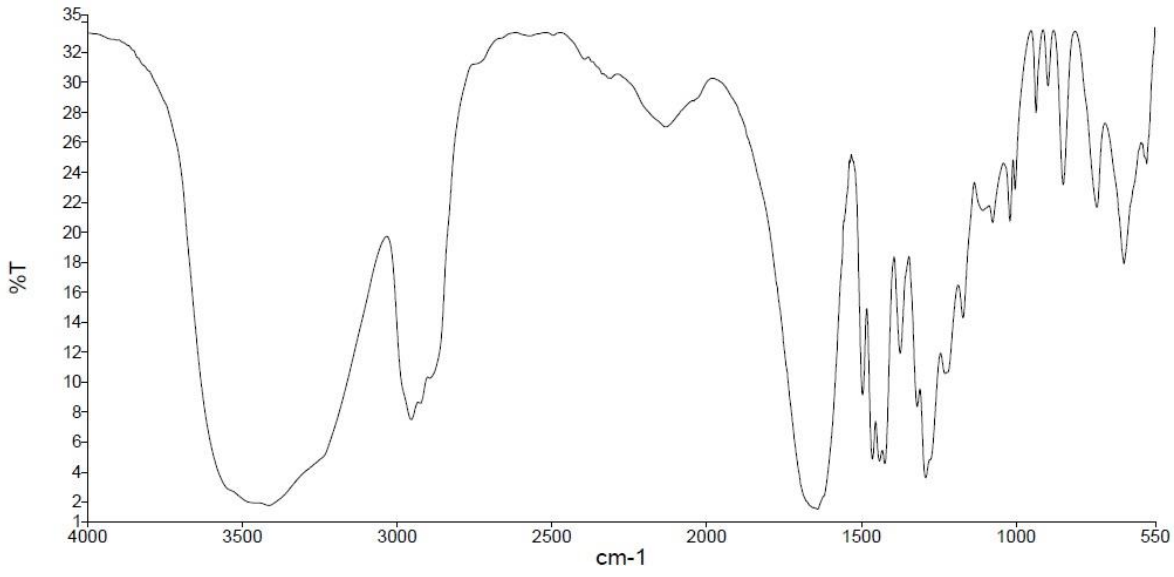

Figure 6.5: IR spectroscopy of poloxamer at scanning range was of 4000-550 cm^{-1} using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.

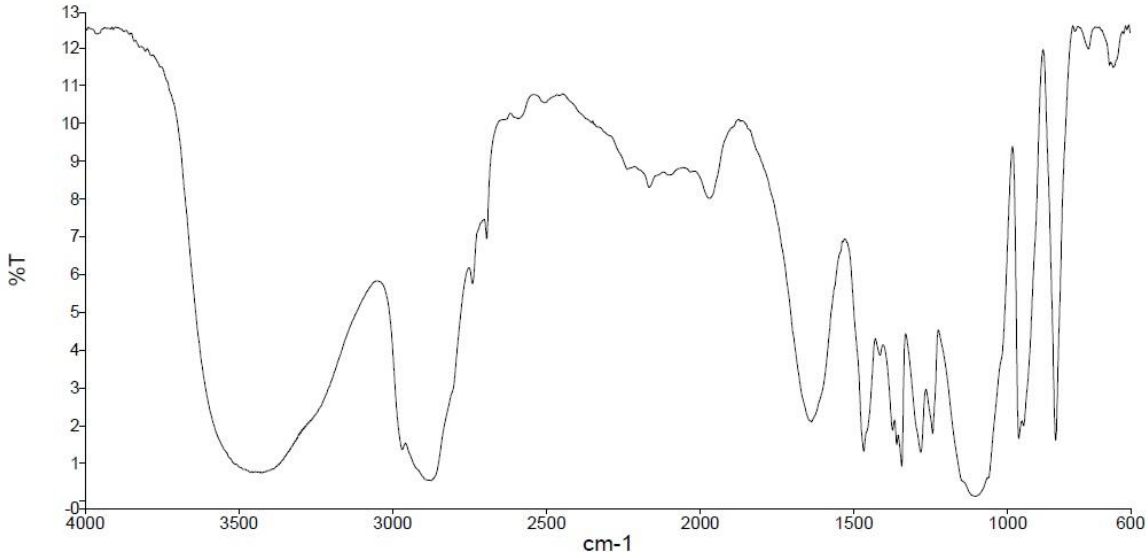

Figure 6.6: IR spectroscopy of PVP K-30 at scanning range was of 4000-550 cm^{-1} using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.

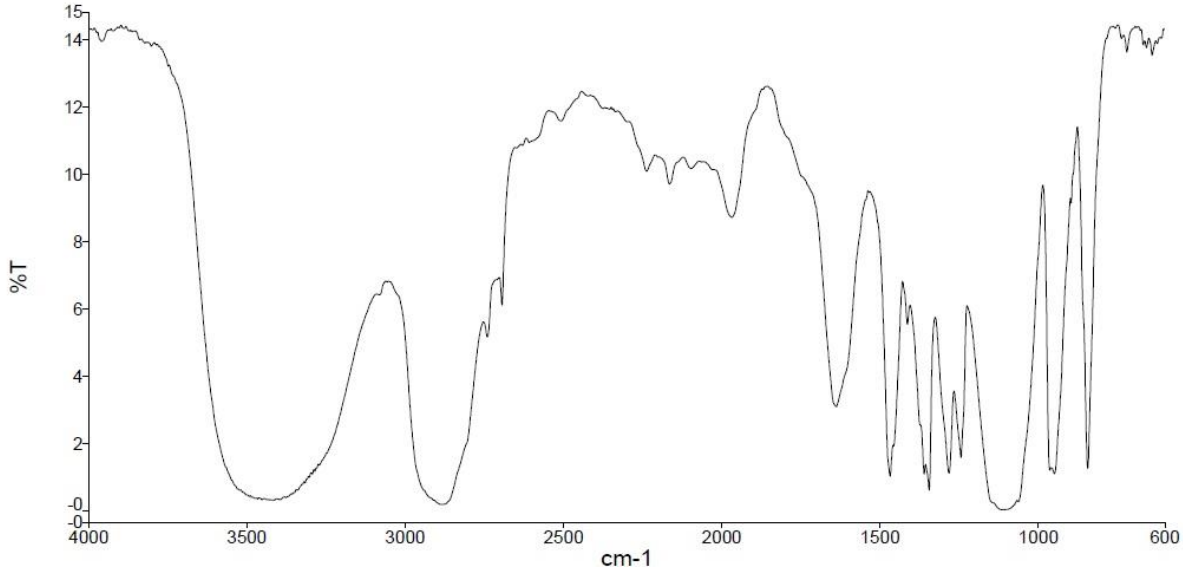

Figure 6.7: IR spectroscopy of HPMCAs at scanning range was of 4000-550 cm^{-1} using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.

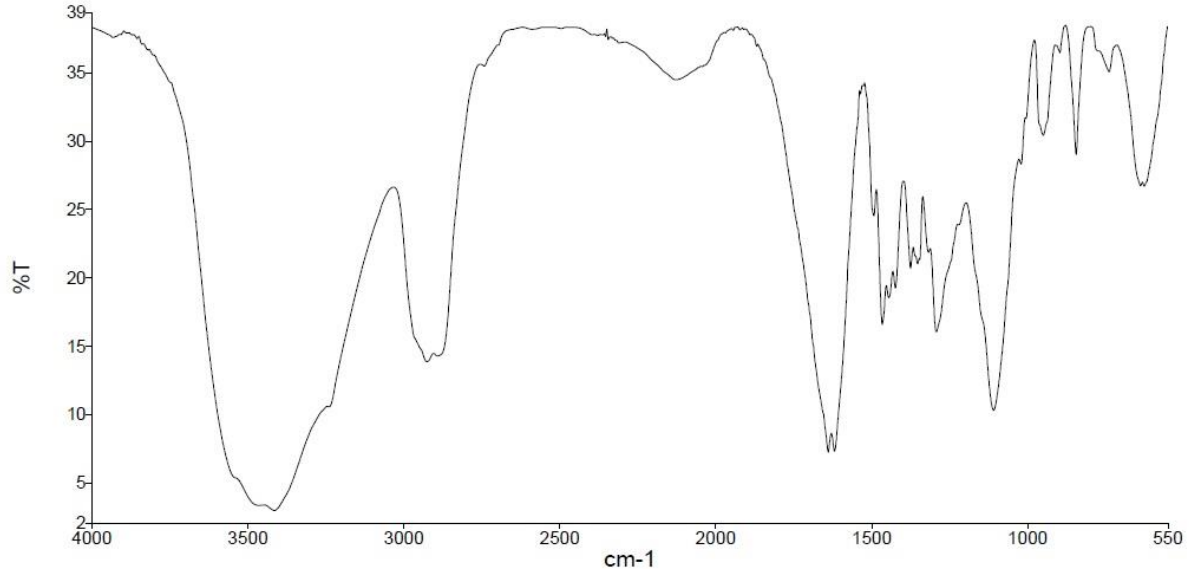

Figure 6.8: IR spectroscopy of Physical mixture of PVP, poloxamer, cholecalciferol and HPMCAS at scanning range was of 4000-550cm^{-1} using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.

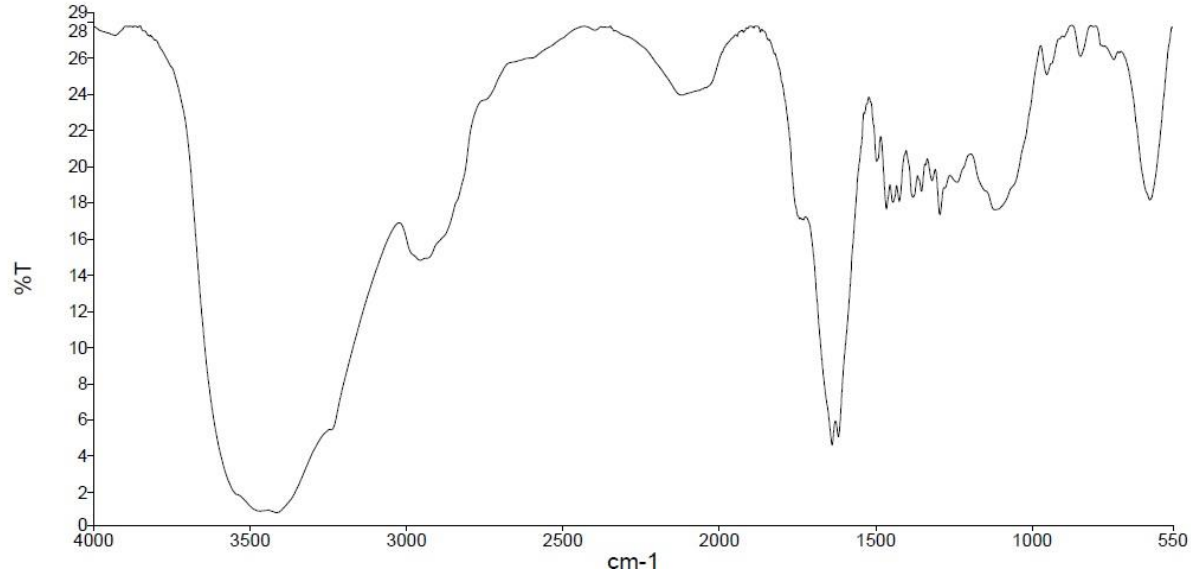

Figure 6.9: IR spectroscopy of solid dispersion formulation CCF-SD-PVP at scanning range was of 4000-550 cm^{-1} using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.

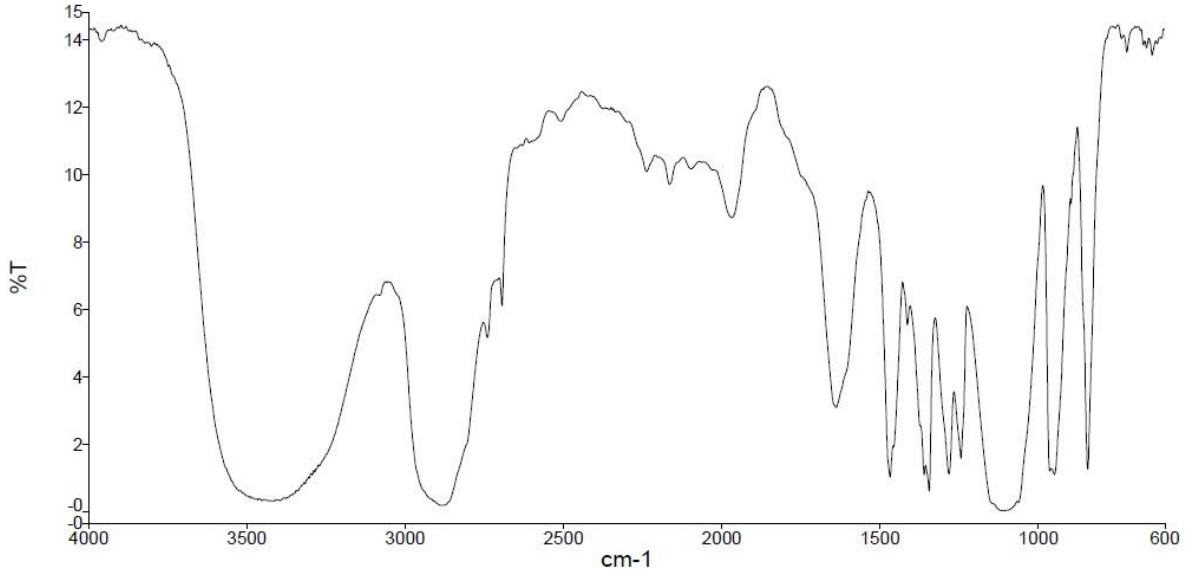

Figure 6.10: IR spectroscopy of solid dispersion formulation CCF-SD-HPMCAS at scanning range was of 4000-550 cm^{-1} using FT-IR instrument (Perkin Elmer Spectrum Two FT-IR Spectrometer version 10.03.06) by KBr pellet method.

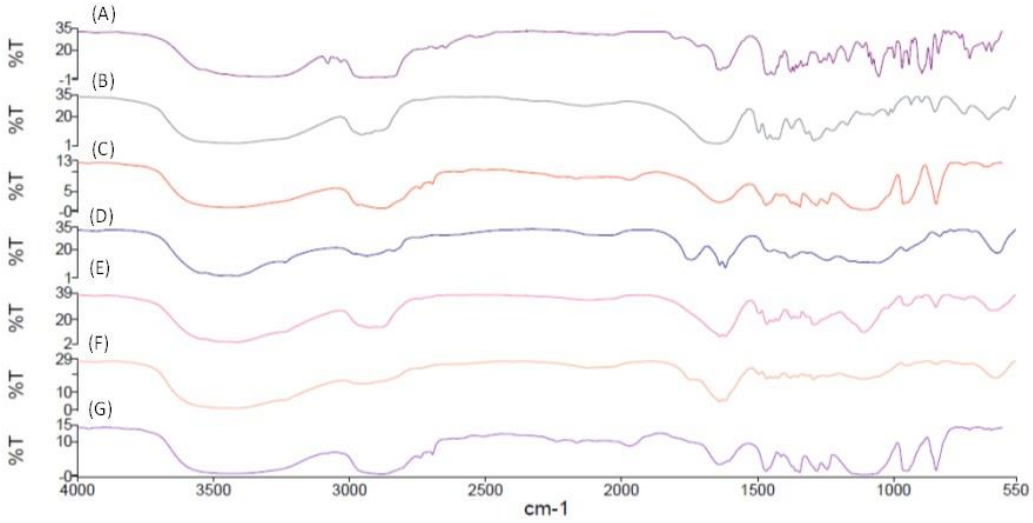

Figure 6.11: IR spectroscopy of solid dispersion formulation, excipients and physical mixture at scanning range was of 4000-550$cm^{-1:}$ (A) Cholecalciferol, (B) Poloxamer, (C) PVP-K30, (D) HPMCAs, (E) Physical mixture of PVP, poloxamer, cholecalciferol and HPMCAS, (F) CCF-SD-PVP, (G) CCF-SD-HPMCAS.

6.3.3 Scanning electron microscopy (SEM)

The surface morphological characteristics of the prepared solid dispersions was investigated by SEM. This study revealed the crystalline form of cholecalciferol with irregular orthorhombic crystals having a clear surface (Fig. 6.12), whereas PVP K-30 and poloxamer exhibited amorphous nature with spherical shaped particles (Fig. 6.13 and Fig. 6.14), and HPMCAs showed irregular shaped particles (Fig. 6.15). The solid dispersion formulations, CCF-SD-HPMCAS (Fig. 6.16), and CCF-SD-PVP (Fig. 6.17), did not reveal any crystal morphology of cholecalciferol and there was a remarkable change in the appearance of polymer. The solid dispersion formulation of cholecalciferol appeared to be small aggregates of amorphous particles. The morphological changes in the particles thus indicate the formation of a new solid phase, correlated with the changes in the crystalline state, leading to a single amorphous phase.

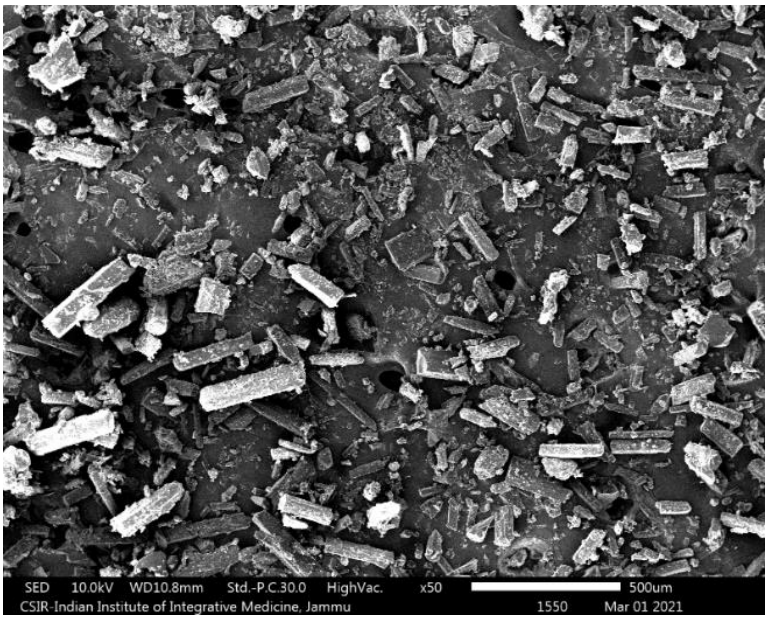

Figure 6.12: Surface morphology of cholecalciferol using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10 kV.

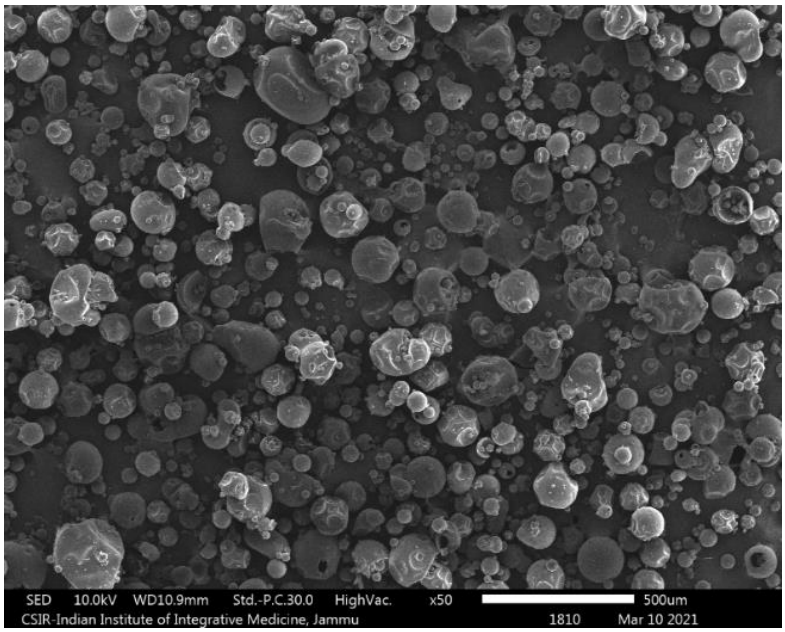

Figure 6.13: Surface morphology of PVP K-30 using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10 kV.

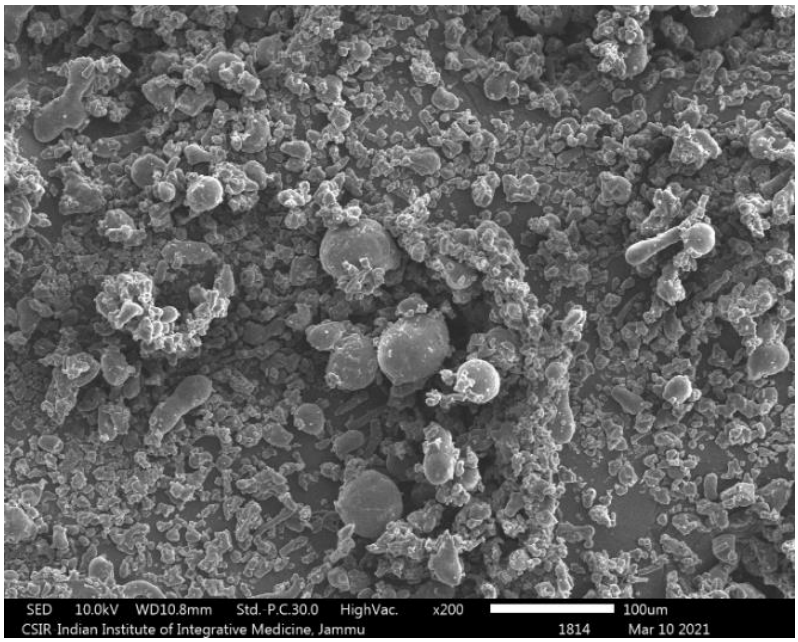

Figure 6.14: Surface morphology of poloxamer using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10 kV.

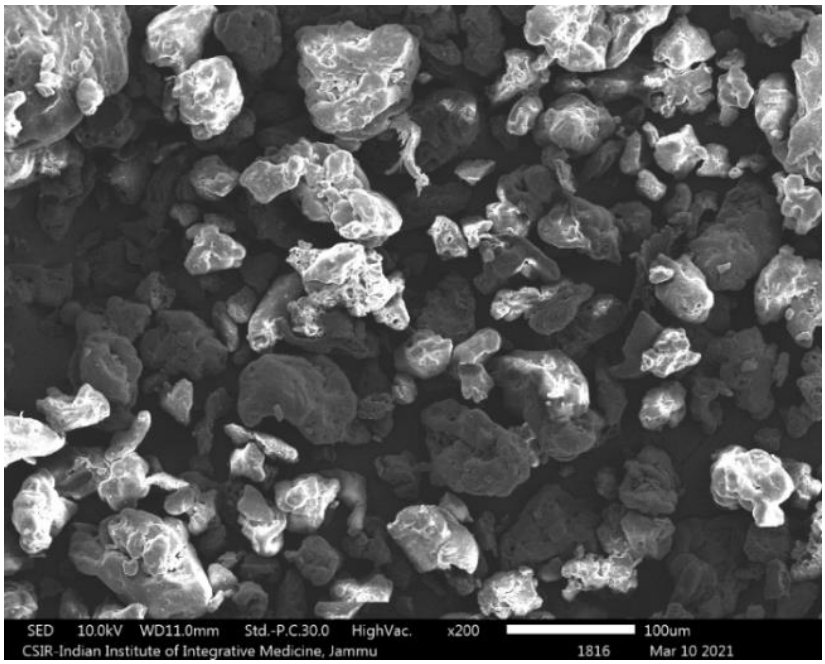

Figure 6.15: Surface morphology of HPMCAS using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10kV.

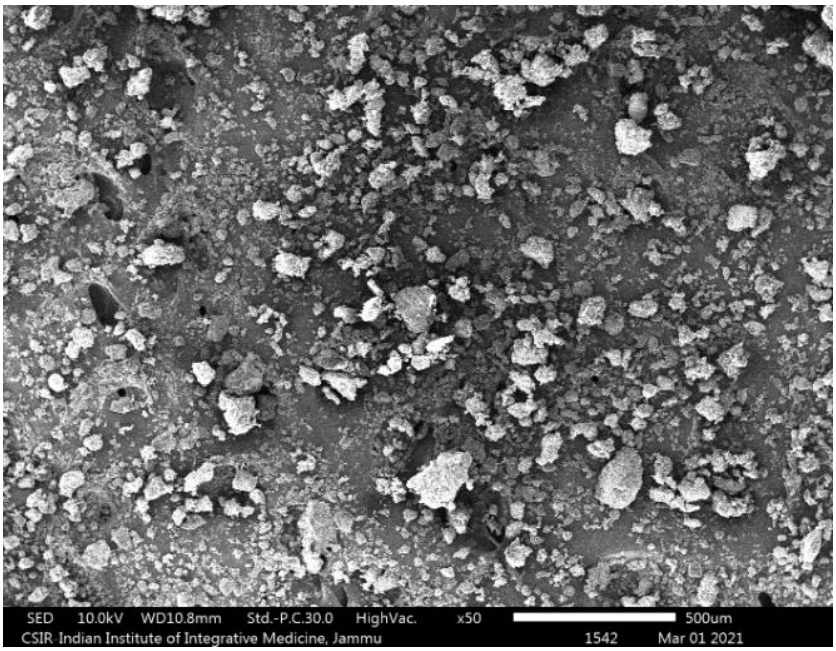

Figure 6.16: Surface morphology of CCF-SD-PVP using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10kV.

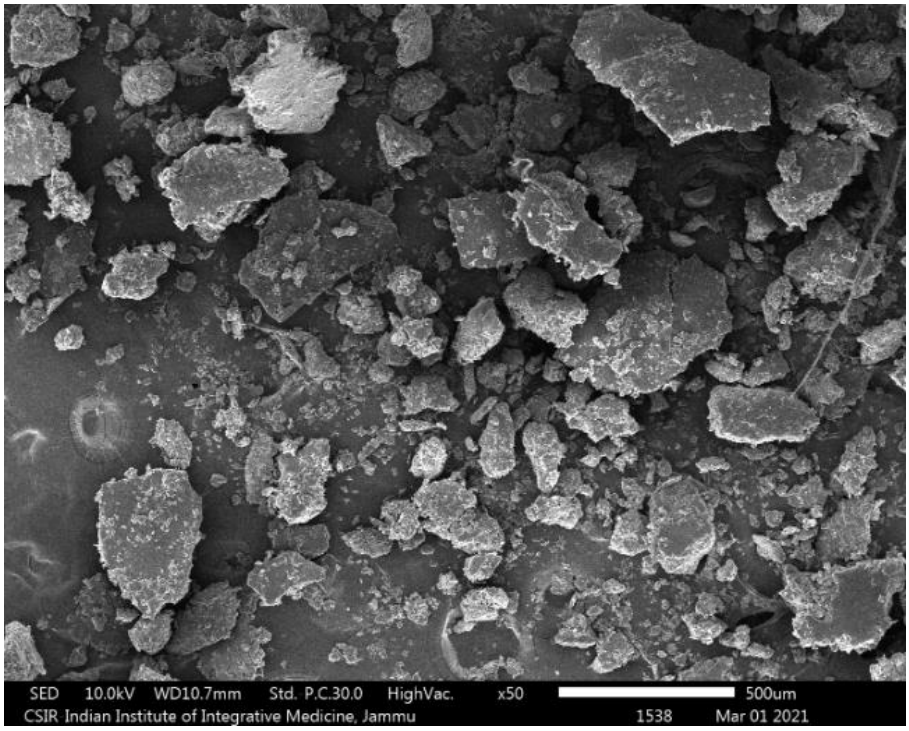

Figure 6.17: Surface morphology of CCF-SD-PVP using scanning electron microscope (JEOL, Japan). Using double-sided carbon adhesive, the experimental specimen was fixed on a brass stub and was sputter coated and image was captured at 10 kV.

6.3.4 Thermal analysis

Differential scanning calorimetry analysis offers an insight into the melting profile of crystalline substances and therefore it provides important information regarding the interaction of drugs with the polymeric materials. The thermal behavior of cholecalciferol can be witnessed by a sharp endothermic peak corresponding to the temperature 85.14˚C (Fig. 6.18) characteristics of its crystalline structure as seen under the SEM. On the other hand, the solid dispersion formulation did not show any endothermic peak event corresponding to the melting of cholecalciferol indicating the formation of solid dispersion.

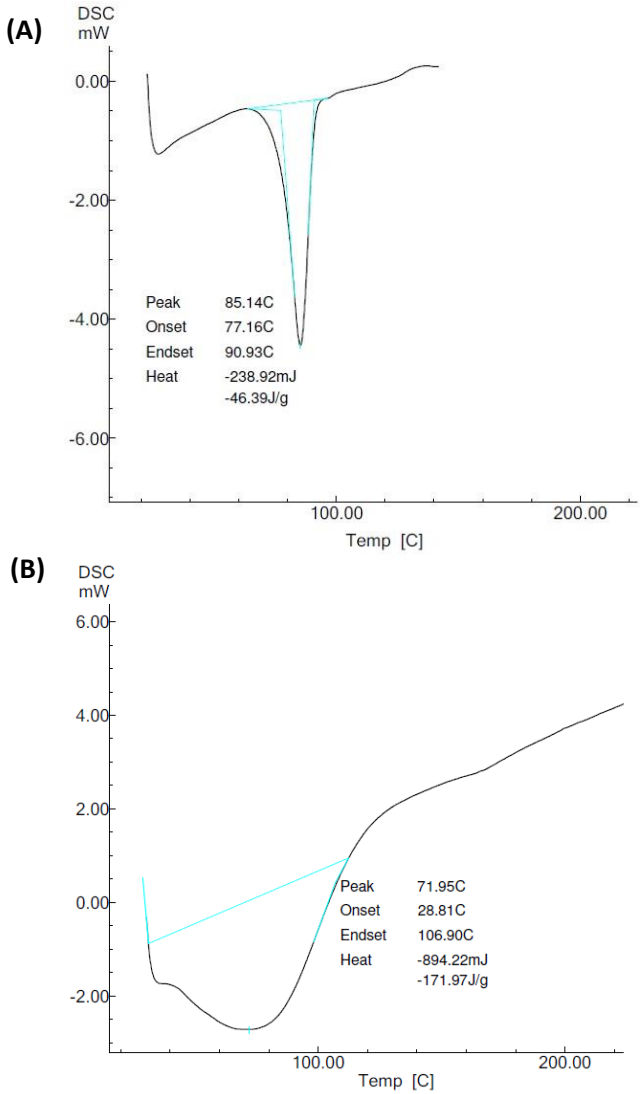

Figure 6.18: Differential scanning calorimetry analysis of (A) Cholecalciferol and (B) Solid dispersion formulation (CCF-SD-HPMCAS) at a scanning rate of 10˚C min^{-1} from 20 to 220˚C.

6.3.5 X-ray diffraction analysis

X-ray diffractograms of cholecalciferol and its solid dispersion formulation (CCF-SD-HPMCAS) at diffraction angle 2θ are shown in Fig. 6.19. Cholecalciferol exhibited crystalline peaks at 2θ = 5.06º, 17.99º, 18.29º and 23.70º attributed to its crystalline characteristics. The findings are in accordance with a previous investigation (Dadkhodazade *et al.*, 2018). On the other hand, in the solid dispersion formulation, the characteristics peaks of cholecalciferol were not appeared. This could be attributed to

the conversion of cholecalciferol in amorphous or molecular dispersion form in the solid dispersion formulations, which is in agreement with an earlier study (Pantić *et al.*, 2016). The amorphous form, owing to higher free energy, is commonly associated with improved solubility compared to the crystalline form (Alonzo, *et al.*, 2010).

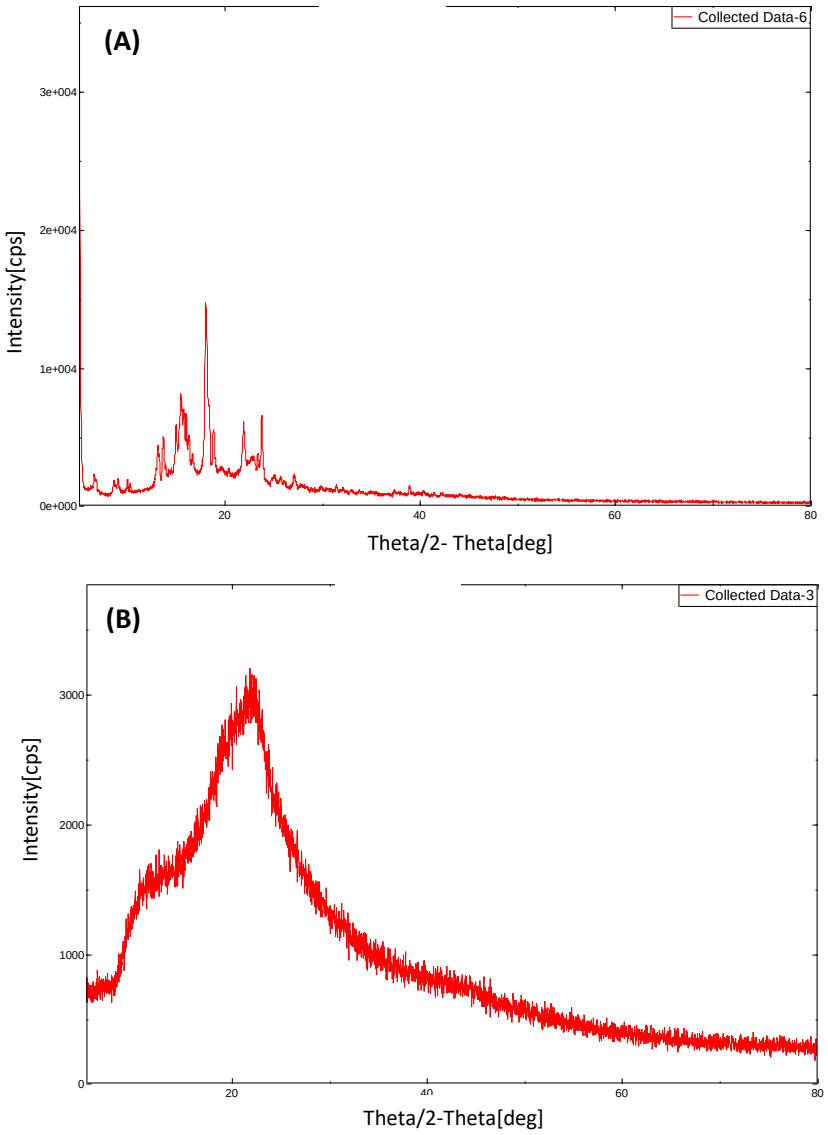

Figure 6.19: X-ray diffraction analysis of powder samples of (A) Cholecalciferol and (B) Solid dispersion formulation (CCF-SD-HPMCAS) at scanning range from 5° to 50° (diffraction angle 2θ).

6.3.6 Effect of formulation on viability of mimics of intestinal cells (caco-2 cells)

Solid dispersion formulation in our study comprises cholecalciferol, polymer and surfactant. Surfactants are commonly used in pharmaceutical formulations comprising

a poorly aqueous soluble active agent. Surfactants could improve drug's solubility via micellar solubilization and play a vital role in the modulation of membrane permeability (Fischer *et al.,* 2011). They also possess the potential to inhibit p-glycoprotein, thereby improving the drug's bioavailability and intracellular drug concentration, which is having an implication in tackling drug resistance. However, surfactants are associated with the limitation of local irritation, membrane disruption, and cellular death. Hence, surfactant-based formulations should be investigated for their cytotoxic potential (Menard *et al.,* 2012) and for this purpose, Caco-2 cells are commonly used. Surfactants can affect Caco-2 paracellular permeability and there are reports corelating paracellular transport of tight junction markers with viability and transepithelial electrical resistance (El-Sayed *et al.,* 2002; Sandri *et al.,* 2007). We have evaluated the effect of various concentrations of cholecalciferol formulations (CCF-SD-HPMCAS and CCF-SD-PVP) on the viability of Caco-2 cells by using MTT assay (Fig. 5.20). It can be seen that the viability of the caco-2 cells with the treatment equivalent to 0.1, 1, 5, 10, and 20 µg/mL of cholecalciferol in the solid dispersion formulation was more than 80% in both formulations (CCF-SD-HPMCAS and CCF-SD-PVP). The results are implying that the polymer/surfactant used in the developed solid dispersion formulation of cholecalciferol had no cytotoxic effect on Caco-2 cells at the indicated concentrations and seems to be nontoxic. The findings are in accordance with a previous study (Mustafa *et al.,* 2022).

6.3.7 Dissolution study

Cholecalciferol is highly susceptible to environmental conditions including light, temperature, and oxygen and can readily be oxidized, causing loss of its functionality and physiological benefits (Gonnet *et al.,* 2010). Moreover, its decomposition is higher

at acidic condition (Gupta *et al.*, 2019). Therefore, enteric polymer (HPMCAS) was used in the present study for the improved delivery of cholecalciferol.

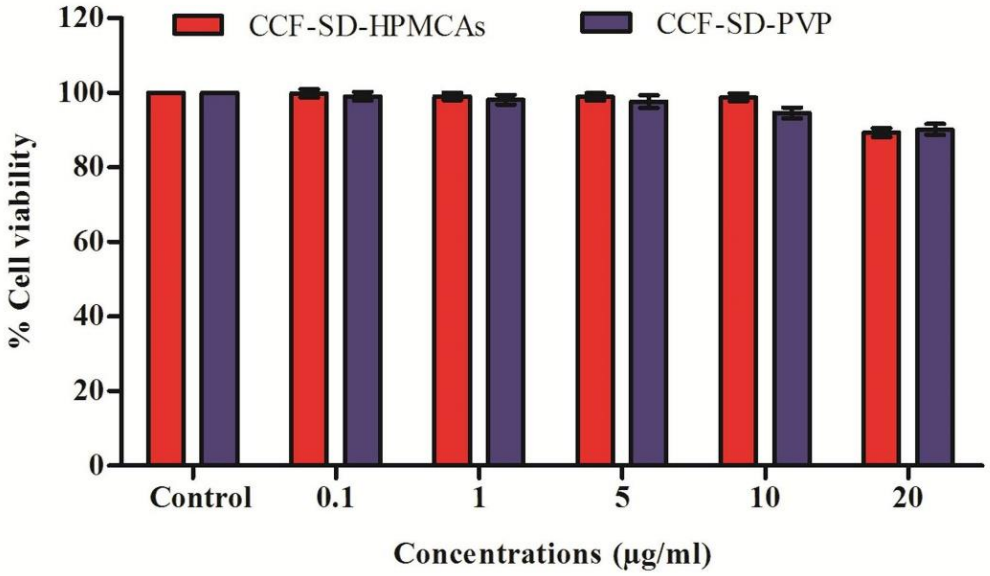

Figure 6.20: Evaluation of compatibility of the formulation with the mimics of intestinal cells. Various concentration of the cholecalciferol formulations (CCF-SD-HPMCAS and CCF-SD-PVP) were incubated with caco-2 cells and the viability of caco-2 cells was assessed by using MTT assay.

In the present study, to better understand the dissolution behavior of cholecalciferol, two-stage dissolution in biorelevant medium was performed. The first stage resembles the environment in gastric fluid and therefore comprises SGF (pH 1.8). The second stage represents the release in intestinal conditions and uses simulated intestinal media (pH 6.8). Cholecalciferol, being lipophilic in nature exhibited poor dissolution in SGF. In SGF, CCF-PVP-SD based formulation showed improved dissolution (22%) compared to the CCF, CCF-PM, and CCF-SD-HPMCAS because HPMCAS is an enteric polymer and dissolves in basic media (Tanno *et al.*, 2004), whereas the solubility of PVP is pH-independent. In CCF-SD-HPMCAS, the polymer acts to prevent cholecalciferol from dissolving, leading to retardation in drug release until the condition of higher pH values is achieved (Fig. 6.21). In SIF, CCF-SD-HPMCAS

significantly improved the dissolution of cholecalciferol (~80%) compared to the CCF, CCF-PM, and CCF-SD-PVP. The improved drug dissolution from CCF-SD-HPMCAS can be due to the decrease in particle size of the drug and possible amorphization of solid dispersion, enhanced wetting of the drug and the possible solubilization effect of the polymer/surfactant. Another mechanism of dissolution enhancement could be complexation of cholecalciferol with hydrophilic PVP macromolecules in water, which is prominent for its intrinsic solubility. PVP forms water-soluble complexes with various small molecules. It additionally, restrict crystallization of dissolved molecules (Kurakula *et al.,* 2020). In addition, the enteric polymer can offer pH-dependent controlled release (Kuramoto *et al.,* 2001; Hasegawa *et al.,* 1986) and improved bioavailability as it restricts recrystallization of cholecalciferol in gastric media (Kai *et al.,* 1996) and drug degradation in acidic conditions.

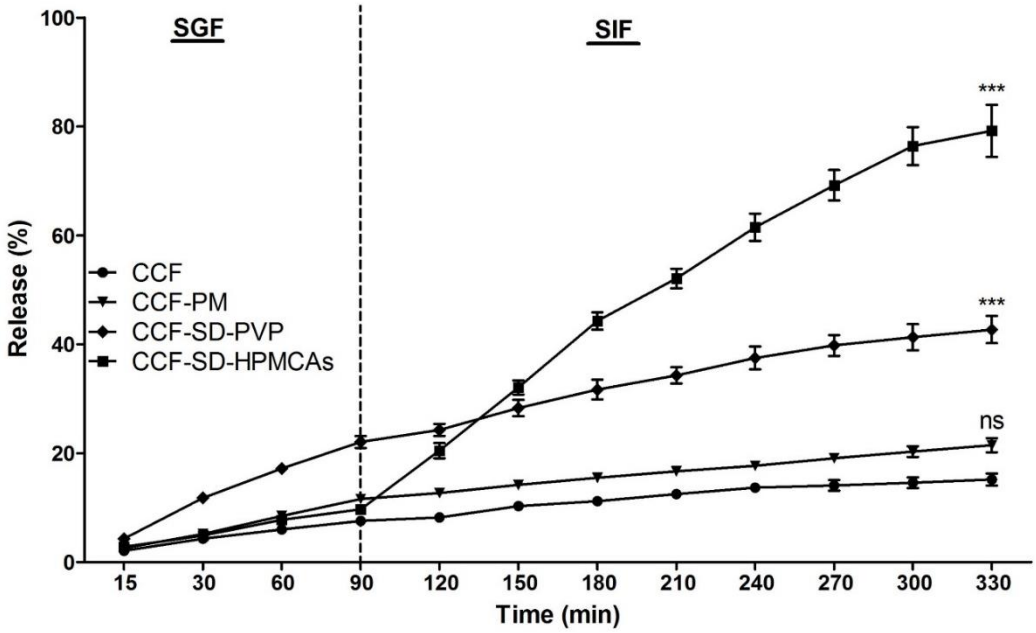

Figure 6.21: Dissolution profile of cholecalciferol (CCF), solid dispersion formulations (CCF-SD-PVP and CCF-SD-HPMCAS), and physical mixture (CCF-PM) in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Dissolution study was conducted for 90 min in SIF and 240 min in SIF using dissolution apparatus with paddle operated at 100 rpm and dissolution medium temperature was kept at 37±0.5˚C.

6.3.8 Relative bioavailability of cholecalciferol

The relative bioavailability of cholecalciferol and its developed formulations were determined in Wistar rat using a previously described method (Ibrahim *et al.*, 2013). The concentration time profile of cholecalciferol (CCF), PVP based solid dispersion (CCF-PVP-SD) and enteric solid dispersion CCF-SD-HPMCAS following oral administration in Wistar rat is shown in Fig. 5.22. The CCF-PVP-SD based formulation showed improved relative bioavailability (3.19 folds) compared to the cholecalciferol (CCF). Further, CCF-SD-HPMCAS based formulation showed marked enhancement in the relative bioavailability (5.62 folds) compared to the cholecalciferol (CCF). The improved relative bioavailability of solid dispersion-based cholecalciferol formulation could be attributed to the better solubility of the cholecalciferol. Further, enteric solid dispersion could offer protection to the cholecalciferol which in turn lead to marked improvement in the relative bioavailability of the cholecalciferol. Moreover, the water-soluble nature of the developed formulation is compatible with the aqueous characteristics of the lining of the gastro-digestive tract, which can also contribute to the improved relative bioavailability of cholecalciferol (Li *et al.*, 2011; Sun *et al.*, 2012).

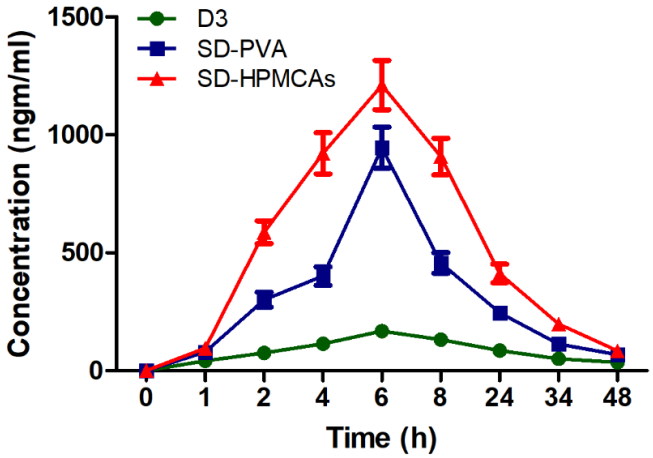

Figure 6.22: The concentration time profile of cholecalciferol (CCF), PVP based solid dispersion (CCF-PVP-SD) and enteric solid dispersion CCF-SD-HPMCAS following oral administration in Wistar rats.

6.3.9 Stability study

Cholecalciferol is an important micronutrient and its stability is susceptible to environmental conditions including light, oxygen, and temperature. Therefore, the stability of the CCF-SD-HPMCAS and CCF-SD-PVP was investigated by storing the formulation at different conditions of temperature and humidity. CCF-SD-HPMCAS and CCF-SD-PVP were stored at 4˚C (refrigerator), 25˚C (60%RH), and 30˚C (65%RH) for three months and samples were analyzed every month for drug content. It was observed that there was a slight decrease in CCF content in CCF-SD-HPMCAS and CCF-SD-PVP after three months at 30˚C (65%RH). However, there was no significant difference in the cholecalciferol content in CCF-SD-HPMCAS (Fig. 5.23). and CCF-SD-PVP after three months at 4˚C and 25˚C/60%RH (Fig. 6.24). The observations are in agreement with a previous study in which a lipid-based formulation improved the stability of calcitriol (Yuan, *et al.,* 2013). The major factors influencing the decrease in cholecalciferol content include temperature, oxygen, acidic conditions, humidity, and light. However, solid dispersion offers immobilization of the cholecalciferol in polymer carriers. Alternatively, the other explanation for the improved stability is the physical barrier for the penetration of oxidizing agents, whose exposure to cholecalciferol is obstructed in the polymeric matrix-based solid dispersion formulation.

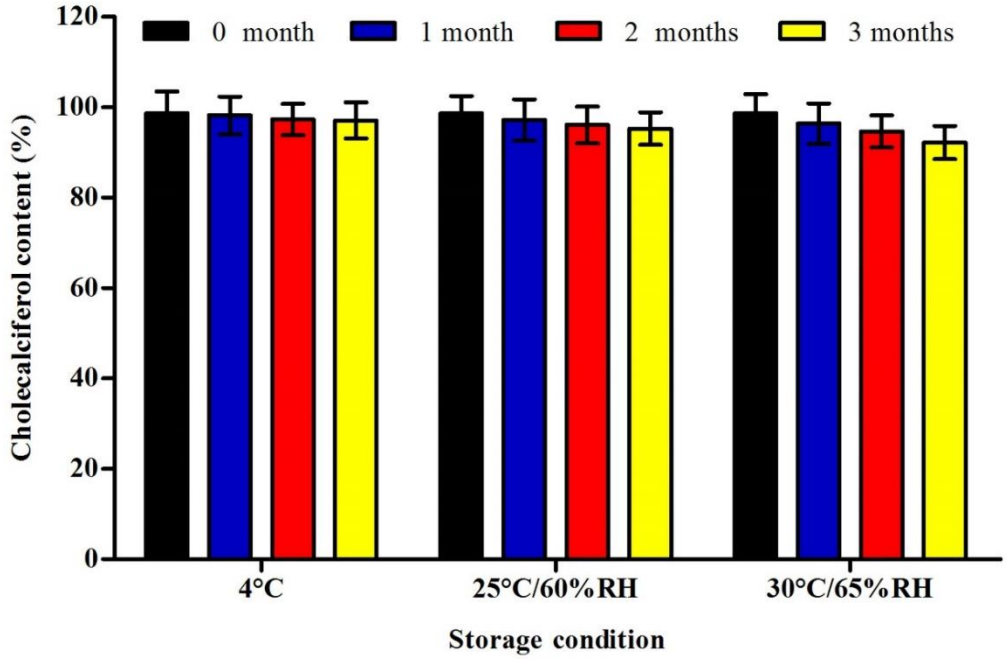

Figure 6.23: Evaluation of change in cholecalciferol content of solid dispersion-based formulation (CCF-SD-HPMCAS) after three months of storage at different conditions (4°C, 25°C/60%RH and 30°C/65%RH).

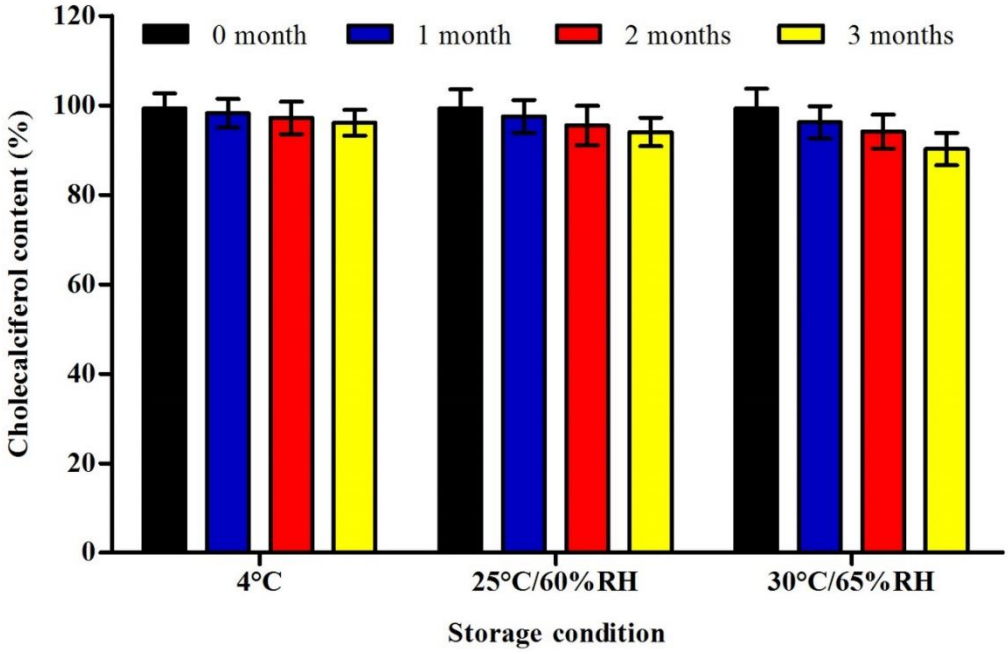

Figure 6.24: Evaluation of change in cholecalciferol content of solid dispersion-based formulation (CCF-SD-PVP) after three months of storage at different conditions (4°C, 25°C/60%RH and 30°C/65%RH).

6.4 Strategy II: Development and Characterization of Delayed Release HPMC capsules for Efficient Delivery of Cholecalciferol Solid Dispersion

6.4.1 Preparation of DRHCap-SD of cholecalciferol

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 minutes 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 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 5.2). 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 6.2) 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. The enhancement of solubility of cholecalciferol from solid dispersion formulation may be due to various reasons including surfactant aided solubilization and improved wetting property, conversion into amorphous form and size reduction.

Table 6.2: Characterization of solid dispersion formulation for DRHCap-SD.

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

6.4.2 FT-IR spectroscopy

FTIR spectroscopy was conducted to investigate any interactions between the cholecalciferol and excipients (Fig. 6.25 and Fig. 6.26). The stretching vibration of hydroxyl group in cholecalciferol can be seen as band at 3411cm^{-1} and 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 (Fig. 6.25). The analysis of spectra of solid dispersion

formulation showed that the characteristics functional group cholecalciferol is retained in the solid dispersion formulation (Fig. 6.26). The study indicated that the structural features of cholecalciferol was not altered during solid dispersion formulation development.

6.4.3 Scanning electron microscopy

The surface characteristics of the developed solid dispersions was examined by scanning electron microscopy. The SEM photomicrograph showed the crystalline nature of cholecalciferol with irregular orthorhombic crystals (Fig 6.27), while PVP K-30 showed amorphous nature with spherical shaped particles (Fig 6.28). The developed solid dispersions formulation (Fig. 6.29) exhibited no crystalline feature of cholecalciferol and formulation appeared as small aggregates of amorphous particles. The changes in the morphology of formulation suggested transitions from crystalline to amorphous phase in the process of obtaining solid dispersions.

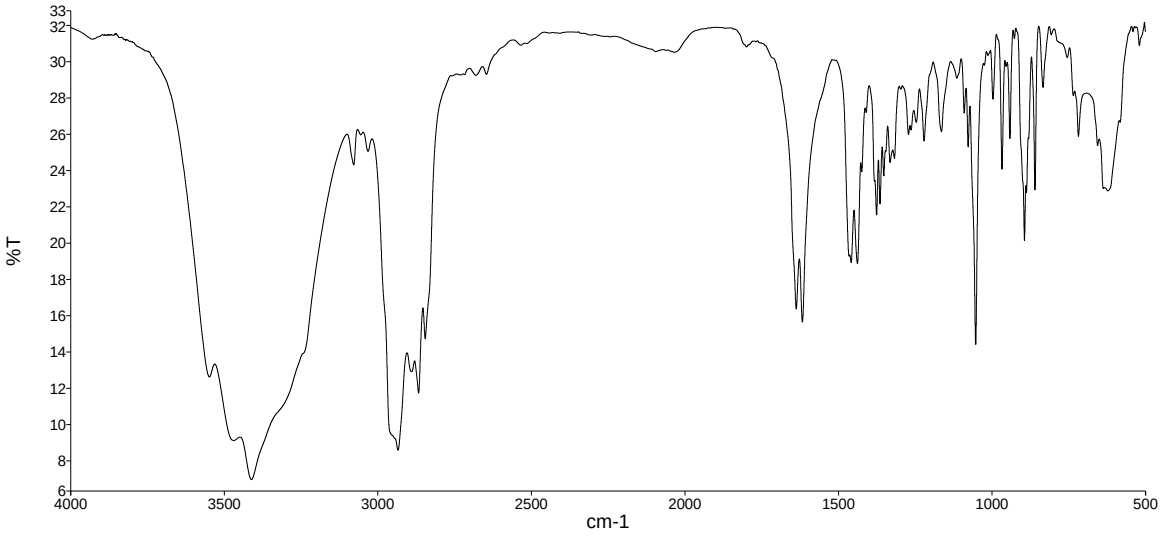

Figure 6.25: IR spectroscopy of cholecalciferol at scanning range of 4000 - 400cm^{-1}.

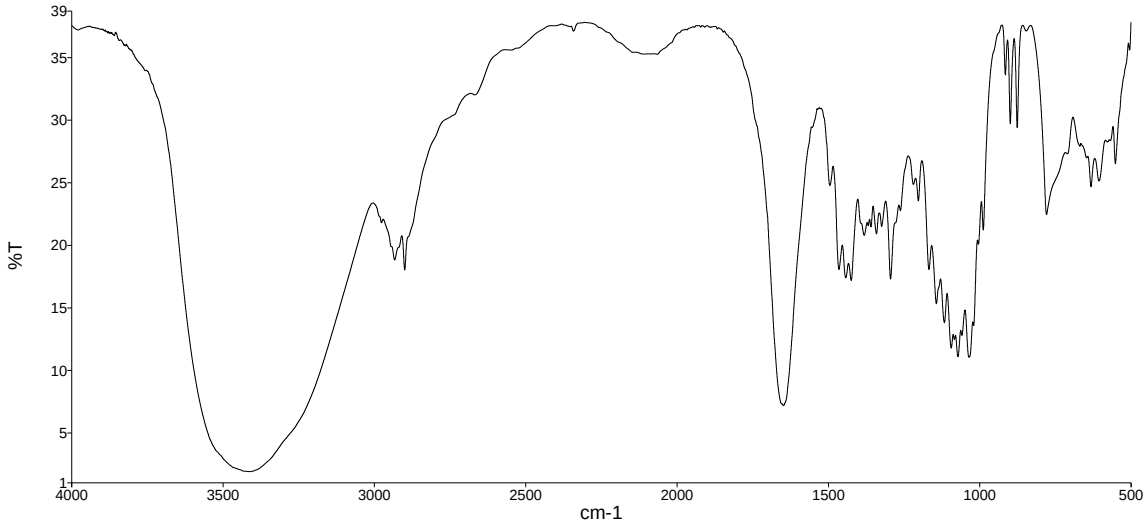

Figure 6.26: IR spectroscopy of solid dispersion formulation at scanning range of 4000-400 cm^{-1}.

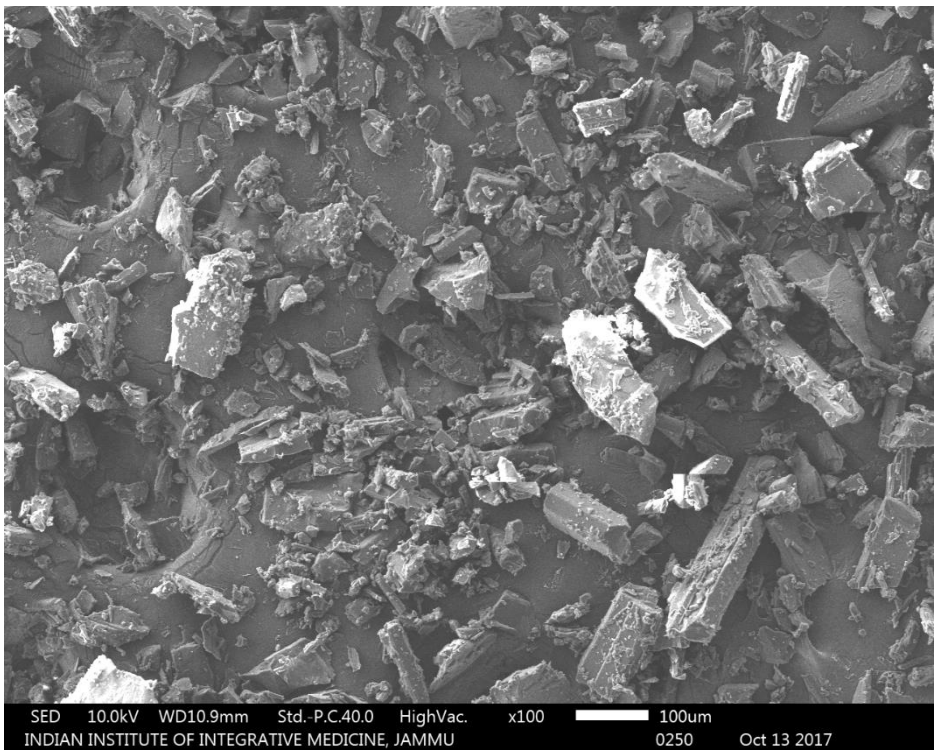

Figure 6.27: Scanning electron microscopic images of Cholecalciferol at accelerating voltage at 10kV.

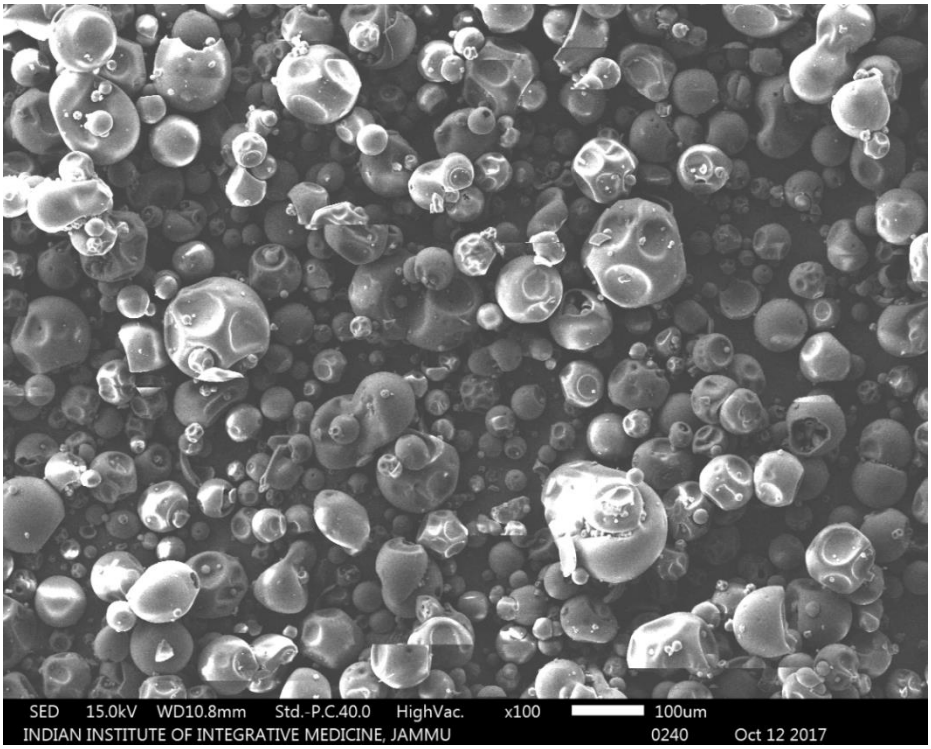

Figure 6.28: Scanning electron microscopic images of PVP-K30 at accelerating voltage at 15kV.

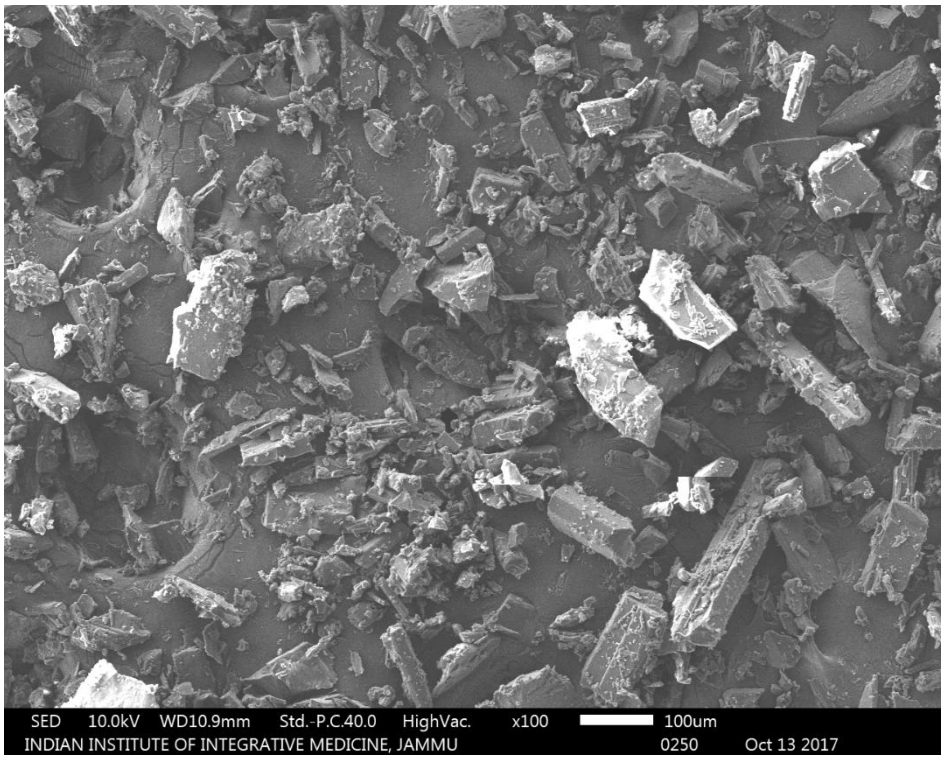

Figure 6.29: Scanning electron microscopic images of solid dispersion formulation (CCF-SD-PVP) at accelerating voltage at 15kV.

6.4.4 Differential scanning calorimetry (DSC) analysis

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 (Fig. 6.30) 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 (Fig. 6.31). Solid dispersion obtained by solvent evaporation technique did not show any endothermic peak event corresponding to melting of cholecalciferol at 92.20 ˚C (Fig.6.32), indicating the formation of solid dispersion where the drug converted from crystalline to amorphous form.

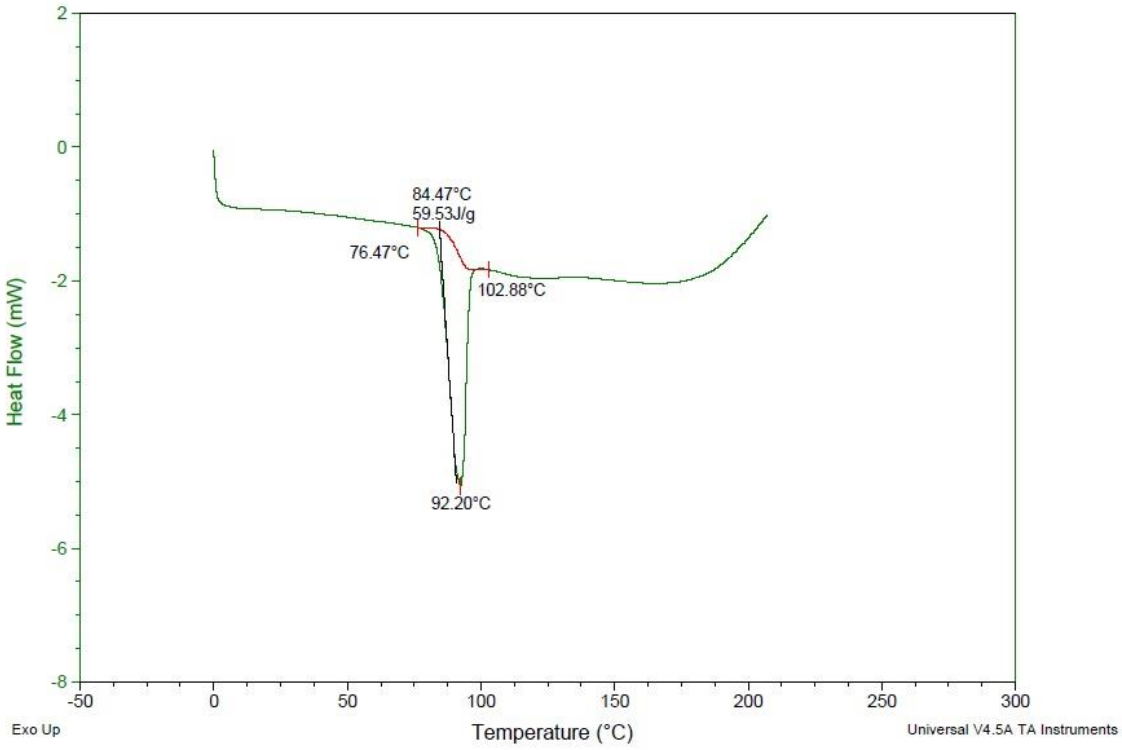

Figure 6.30: Differential scanning calorimetry analysis of cholecalciferol at a scanning rate of 10°C min^{-1} from 20 to 220°C.

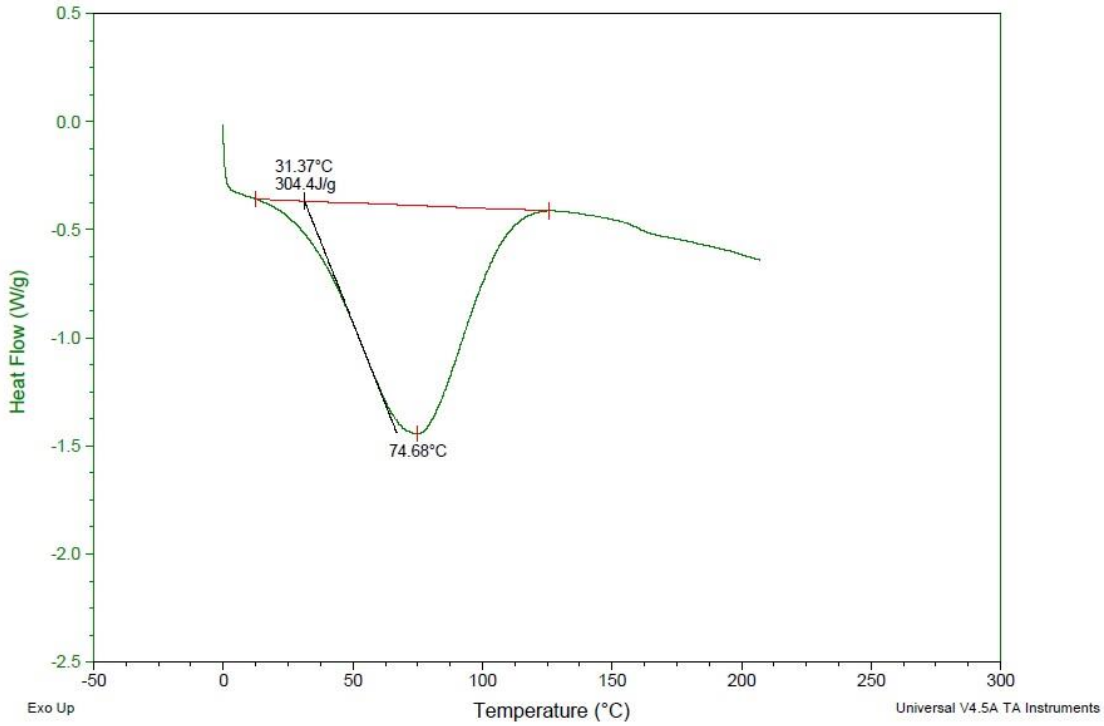

Figure 6.31: Differential scanning calorimetry analysis of PVP K-30 at a scanning rate of 10°C min^{-1} from 20 to 220°C.

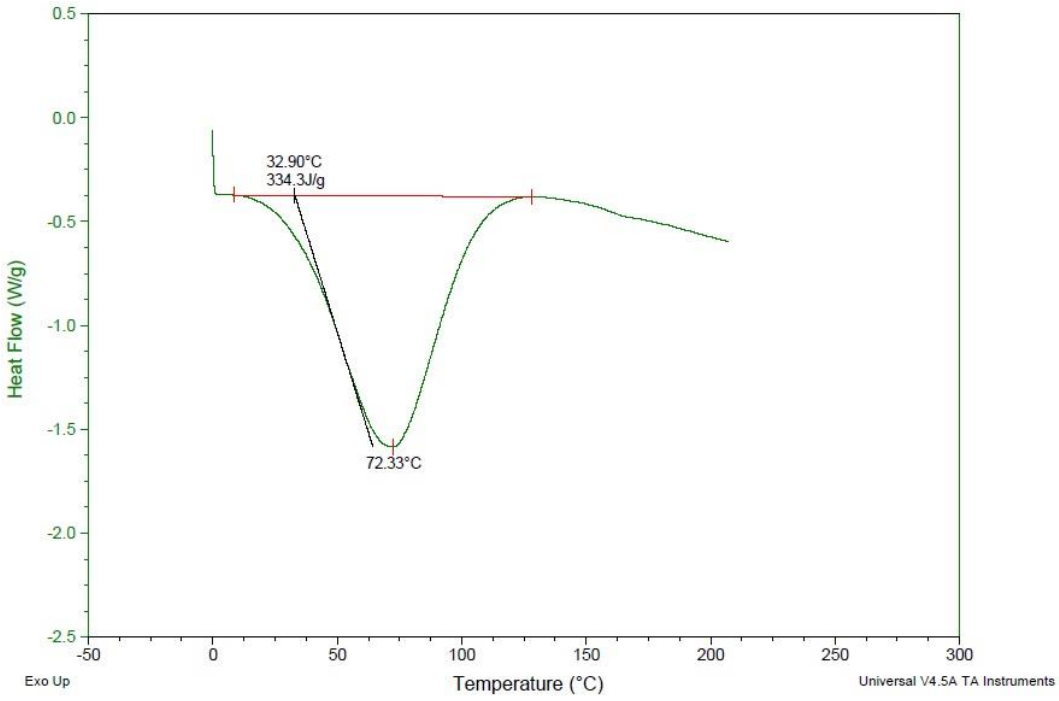

Figure 6.32: Differential scanning calorimetry analysis of solid dispersion formulation (CCF-SD-PVP) at a scanning rate of 10°C min^{-1} from 20 to 220°C.

6.4.5 X-ray diffraction analysis

Cholecalciferol and its solid dispersion formulation (CCF-SD-PVP) was characterized using X-ray diffraction analysis at diffraction angle 2θ (Fig. 5.33.) Cholecalciferol exhibited crystalline peaks at 2θ = 5.18º, 13.69º, 15.87º, 18.15º and 18.81º due to its crystalline characteristics. This observation is in accordance with a previous study (Dadkhodazade *et al.,* 2018). On contrary, the characteristics peaks of cholecalciferol were not appeared in the solid dispersion formulation (Fig. 6.34). The reason could be the change in the crystalline characteristics of cholecalciferol to amorphous nature, which is supported by an earlier report (Pantić *et al.,* 2016). The amorphous form of the molecule is normally associated with the feature of higher solubility as compared to the crystalline form (Alonzo *et al.,* 2010).

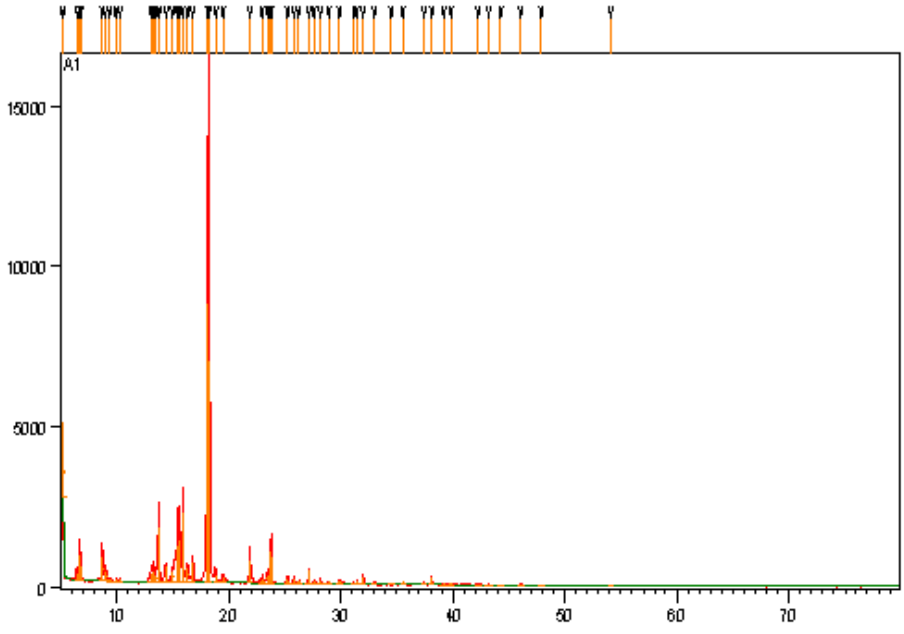

Figure 6.33: X-ray diffraction analysis of cholecalciferol at scanning range from 5° to 80° (diffraction angle 2θ).

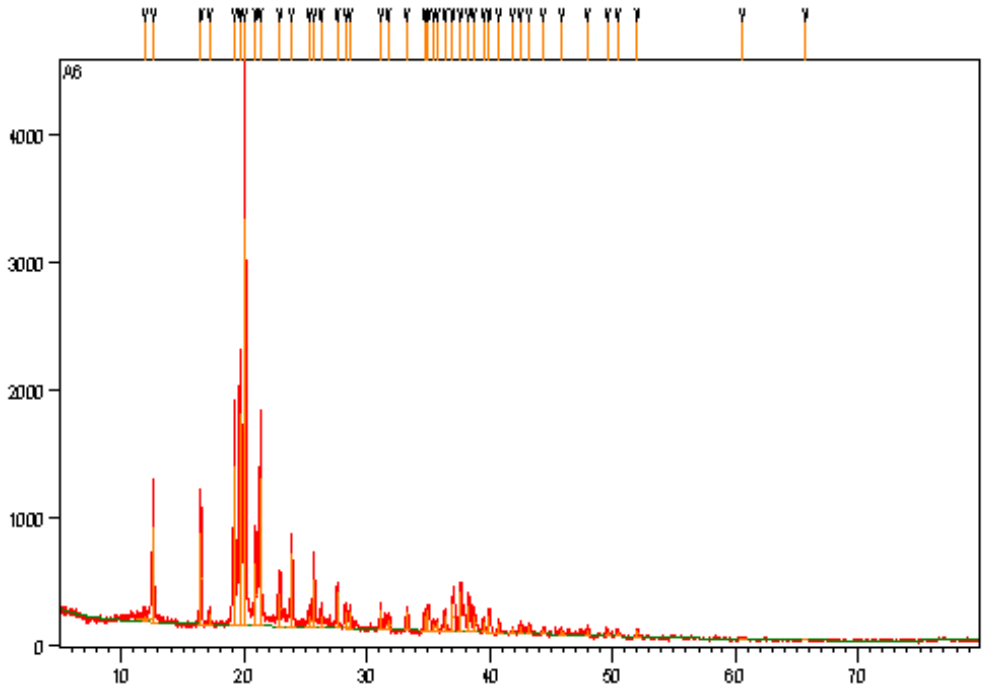

Figure 6.34: X-ray diffraction analysis of solid dispersion formulation (CCF-SD-PVP) at scanning range from 5° to 80° (diffraction angle 2θ).

6.4.6 Effect of formulation on viability of Caco-2 cells

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 (Fischer *et al.,* 2011). However, surfactants are notorious for their local irritation effect, membrane disruption, and cellular death. Therefore, surfactant containing compositions should be assessed for cellular toxicity (Menard *et al.*, 2012) 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 (El-Sayed *et al.*, 2002; Sandri *et al.*, 2007). 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 (Fig. 6.35). 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 (Mustafa *et al.*, 2022).

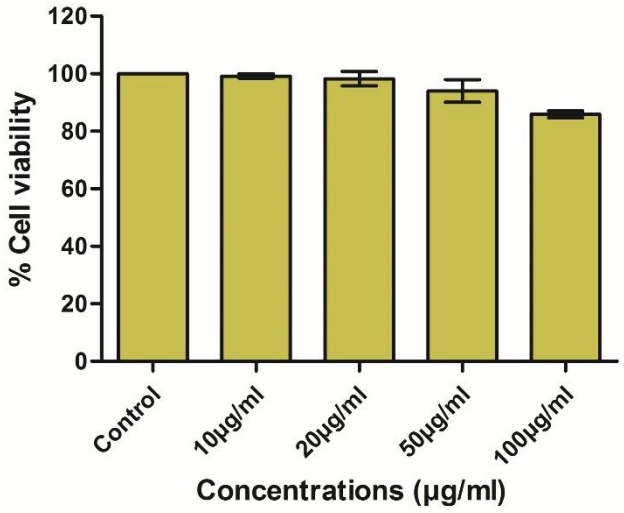

Figure 6.35: Evaluation of compatibility of the formulation with caco-2 cells. Various concentration of the cholecalciferol solid dispersion-based formulations was incubated with caco-2 cells and the viability of caco-2 cells was assessed by using MTT assay.

6.4.7 *In vitro* dissolution

Cholecalciferol is an important micronutrient and it has implication in various cellular events required for normal functioning of the body. This lipophilic agent is very sensitive to the environmental conditions (Gonnet *et al.,* 2010) and its degradation rate is more at low pH (Gupta *et al.*, 2019). 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 simulated intestinal fluid (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 (Fig.6.36). 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). 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.

6.4.8 Flow properties

The bulk properties of the developed solid dispersion and their physical mixture were determined and compared with the USP specifications (Carr RL, 1965). The angle of repose of CCF-SD-PVP and its physical mixture was good (Table 6.3). 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. The results demonstrated that the flow properties were improved by formulation of solid dispersion.

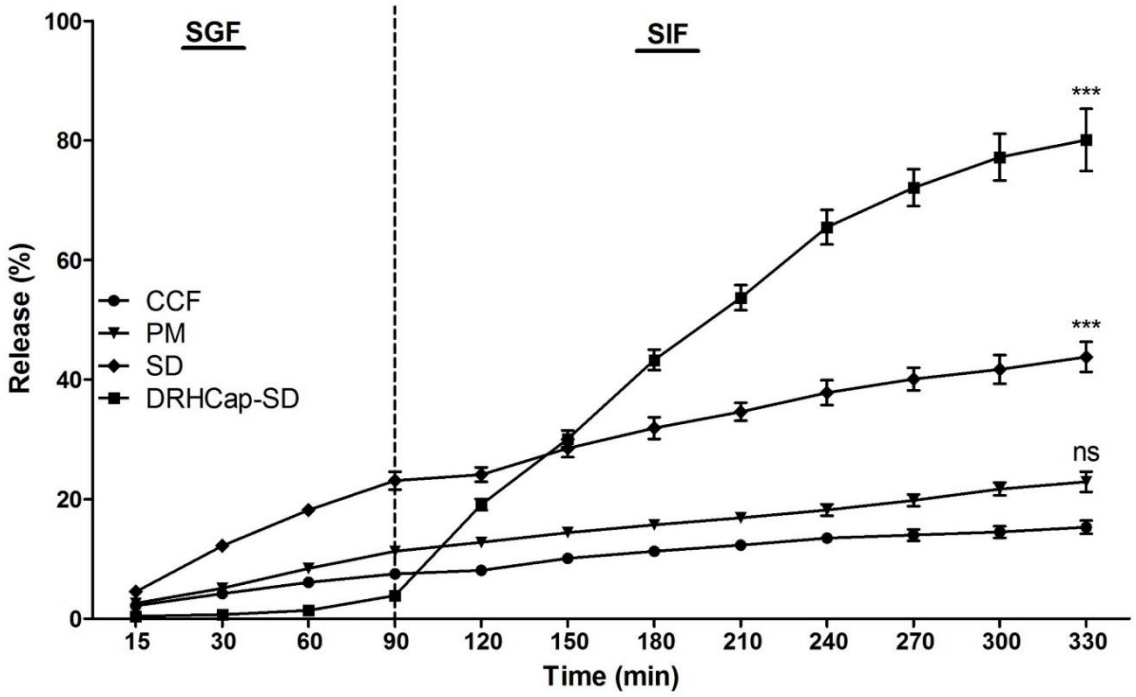

Figure 6.36: Dissolution profile of cholecalciferol (CCF), physical mixture (PM), solid dispersion (SD), and solid dispersion in delayed release HPMC capsule (DRHCap-SD) in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Dissolution study was conducted for 90 min in SGF and 240 min in SIF using dissolution apparatus with paddle operated at 100 rpm and the dissolution medium temperature was kept at 37±0.5°C.

Table 6.3: 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

6.4.9 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 cholecalciferol content. The results showed that there was a decrease in CCF 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 (Fig. 6.37 and Fig 6.38). These findings are in accordance to a previous report which demonstrated improved the stability of calcitriol with a lipid-based formulation (Yuan *et al.,* 2013). Also, a polymeric formulation demonstrated good stability profile of cholecalciferol at different conditions of storage (Vora *et al.,* 2017). 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.

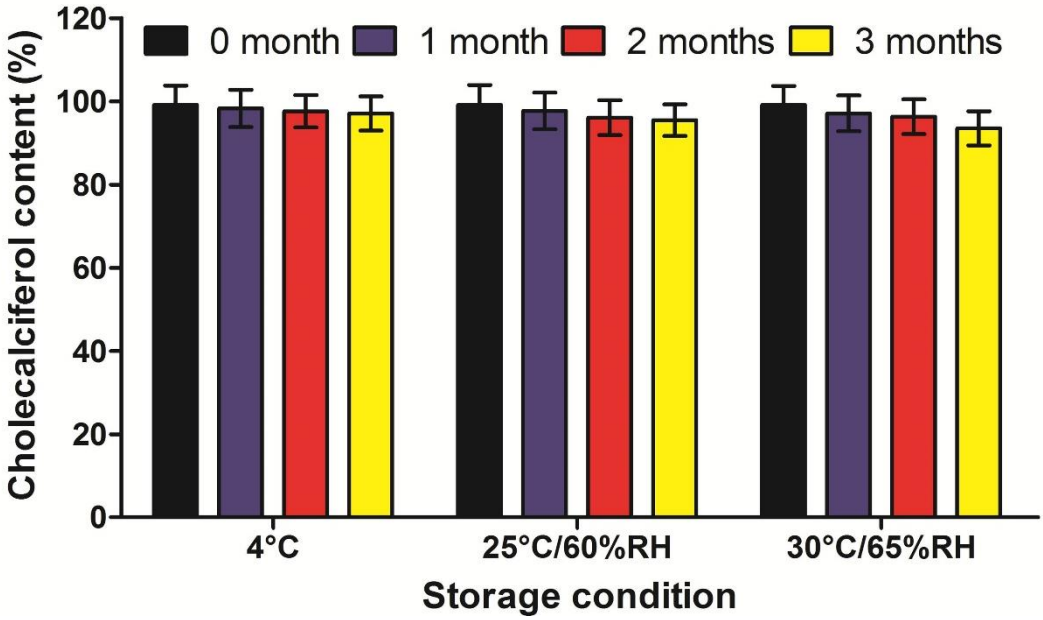

Figure 6.37: Evaluation of change in cholecalciferol content of solid dispersion-based formulation (DRHCap-SD) after three months of storage at different conditions (4°C, 25°C/60% RH and 30°C/65% RH).

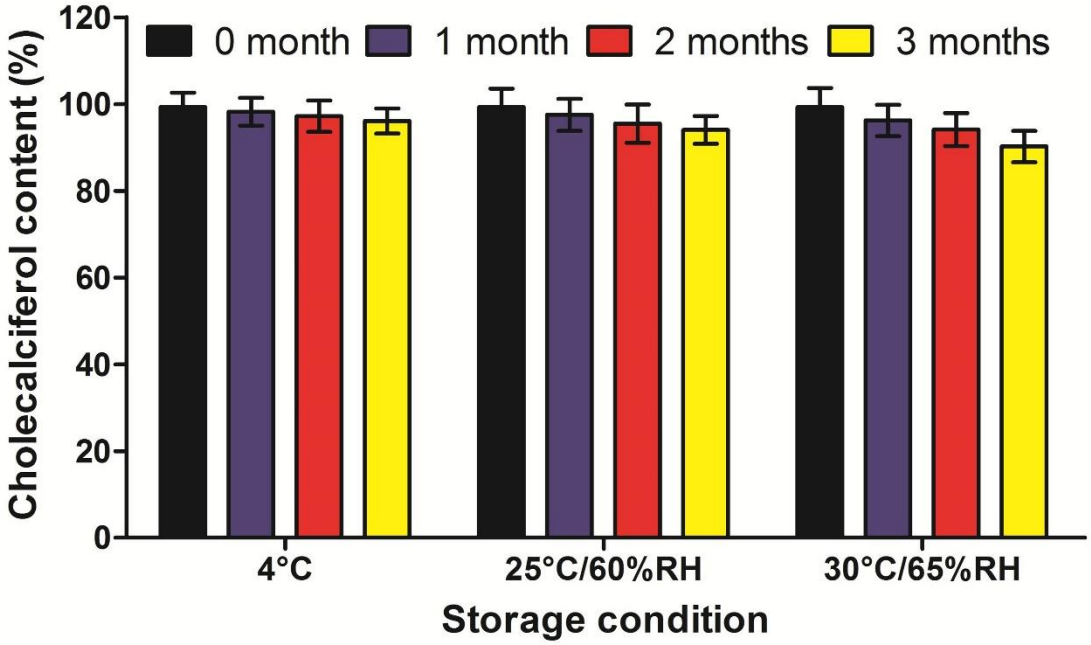

Figure 6.38: Evaluation of change in cholecalciferol content of solid dispersion-based formulation (SD) after three months of storage at different conditions (4°C, 25°C/60%RH and 30°C/65%RH).

6.5 Strategy III: Development and Characterization of Self-emulsifying Drug Delivery Systems for Cholecalciferol

6.5.1 Solubility in various excipients

The composition of the SEDDS formulation should be simple, safe and compatible with the active agent. In addition, it should offer good solubility for intended pharmacological agent. Self–emulsifying drug delivery system comprise of oil, surfactant, and co-surfactant and therefore it exhibited excellent solubility of the bioactive in the formulation composition. The solubility of cholecalciferol in different solvents is presented in Table 6.4. Among the solvents investigated oleic acid exhibited higher solubility of cholecalciferol (39.5±1.1 mg/ml). Also, Cremophore and polyethylene glycol 400 showed higher solubility for cholecalciferol compared to other surfactants. Therefore, in the present study oleic acid was chosen as oil phase whereas Cremophore was taken as surfactant and polyethylene glycol 400 was chosen as co-surfactant.

Table 6.4: Solubility of cholecalciferol in various excipients

Component of SEDDS	Vehicle	Solubility (mg/mL)
Oil	Soya oil	18.4±0.9
	Oleic acid	39.5±1.1
Surfactant	Cremphore	15.4±0.8
	Tween 80	12.8±0.8
Co-surfactant	PEG 400	8.1±0.4
	Propylene glycol	7.5±0.5

6.5.2 Preparation of pseudo-ternary phase diagram

The use of ternary phase diagram is to find out the suitable concentration of various excipients which can yield a homogenous pre-concentrates offering attributes of self-emulsification and optimum drug loading (Anette *et al.,* 2010). Oleic aicd was chosen

as oil phase and its concentration at three range 70%, 65%, and 60% . The concentration of surfactant cremophor EL and co-surfactant polyethylene glycol was optimized while keeping oil concentration fixed. The area in the plot for the microemulsion regions was selected. In the phase diagram the wider region showed the better self-emulsification property of selected composition. A series of self-emulsifying systems with combinations of oleic acid (60-70%w/w), Cremophor (5–40%, w/w), and polyethylene glycol 400 (5–35%, w/w) was prepared to see the effect of oils on the microemulsion formation (Table 6.5). The prpared SEDD formulation was characteried for tendency to form emulsion and phase separation at 5min and 60 min end points. The density and uniformity of globule was also noted.

Table 6.5: Various composition of SEDDS for pseudo-ternary phase diagram.

Oleic acid (%)	Cremophor (%)	PEG (%)	Tendency to form emulsion	Phase separation		Density of globules	Uniformity in globules
				5 min	60 min		
70	30	0	Bad	Yes	Yes	+	+
70	25	5	Bad	Yes	Yes	+	+
70	20	10	Good	No	No	++	++
70	15	15	Good	No	No	++++	++++
70	10	20	Good	No	No	+++	+++
70	5	25	Bad	No	Yes	+	+
65	35	0	Bad	Yes	Yes	+	+
65	30	5	Good	Yes	Yes	+++	+++
65	25	10	Good	No	No	++	+++
65	20	15	Good	No	No	+	+++
65	15	20	Good	No	No	+++	+++
65	10	25	Good	No	Yes	+++	+++
65	5	30	Bad	Yes	Yes	+	+
60	40	0	Bad	Yes	Yes	+	+
60	35	5	Bad	Yes	Yes	+	+
60	30	10	Bad	No	Yes	+	+
60	25	15	Good	No	Yes	++	++++
60	20	20	Good	No	No	++	++++
60	15	25	Good	No	No	++++	++++
60	10	30	Good	No	No	+++	++++
60	5	35	Good	No	Yes	++	++++

The ternary phase diagram was constructed to find out the self emulsification area and to select the ratio of oil, surfactant and cosurfactant in the SEDDS preparation. The ternary phase diagram of the composition comprising Oleic acid as oil phase, Cremophore EL as surfactant and polyethylene glycol as co-surfactant is shown in Fig 6.39.

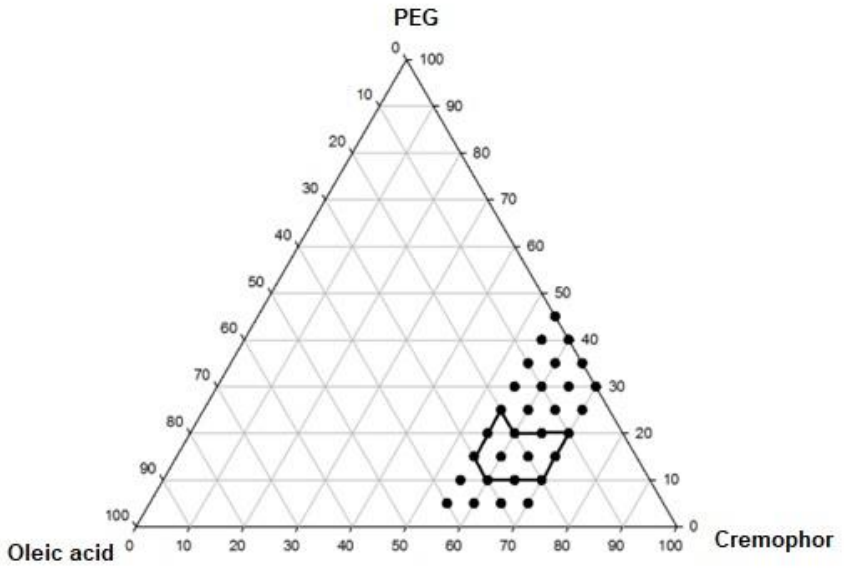

Figure 6.39: Pseudo-ternary phase diagram comprising oleic acid, Cremophor and polyethylene glycol

6.5.3 Characterization of self-emulsifying drug delivery system

On the basis of phase diagram, three points from good emulsification region was selected and formulation compositions corresponding to these points were designated as SF-1, SF-2 and SF-3. The concentration of oil, surfactant and co-surfactant in the SF-1, SF-2 and SF-3 is given in Table 6.6. These three formulations were again characterized on the basis of emulsification tendency, phase separation, density of globules and globules uniformity.

6.5.3.1 Assessment of self-emulsification

The SEDDS formulations were characterized for tendency for micro-emulsification via dilution of the SEDDS pre-concentrate with deionised water. After suitable time interval the dispersion was evaluated for the time of self-microemulsification, appearance, and uniformity and density of globules by observing under the microscope (Table 5.7) and scored according to the grading system shown in Table 5.6 (Craig D.Q.M., 1995; Kommuru, *et al.*, 2001).

Table 6.6: Grading system for assessing emulsification of formulation

No emulsification	Bad
Droplets settled on the bottom of the beaker	Bad
Droplets formed on the top of the beaker, gentle stirring led to droplet to further spread	Moderate
Droplets Spread in the beaker /Gentle stirring led to droplet emulsification	Good
Uniformity in size of emulsion droplets (seen on microscope)	'+'grading
Density of emulsion droplets	'+'grading

Table 6.7: Evaluation of self-emulsification

Formulation code	Emulsification tendency	Phase separation	Globule density	Globule uniformity	Drug precipitation
SF-1	Good	No	+++	+++	No
SF-2	Good	No	++++	++++	No
SF-3	Good	No	+++	+++	No

6.5.3.2 Morphological examination by transmission electron microscopy

The formulation composition of SF-2 exhibited optimum characteristics as shown in Table 6.7 and therefore selected for further studies. The formulation SF-2 turned into

emulsion following dispersion in distilled water and exhibited spherical morphology under the transmission electron microscope (Fig. 6.40).

6.5.3.3 Determination of emulsion particle size and zeta potential

The size of globule in self-emulsified drug delivery systems is of particular importance as it profoundly affect the rate and extend of bioactive release which in turn could have impact on the potential for absorption. Therefore, the determination of the size of globule is an important aspect for self-emulsifying drug delivery formulation. A common phenomenon observed in the case of self-emulsifying drug delivery system is that higher surfactant concentration leads to a reduction in the mean droplet size. This could be attributed to the stabilization of the oil droplets owing to the localization of the surfactant molecules at the oil- water interface. It is believed that smaller the droplet size could offer the larger the interfacial surface area for improved drug absorption (Ping Zhang, *et al.,* 2008; Gershanik and Benita, 2000). Droplet size analysis was performed using dynamic light scattering technique. The optimum SEDDS formulation (SF-2) showed size, polydispersity index and zeta potential as 281.4±11.4 nm, 0.125 and -22.4±1.4, respectively (Fig. 6.41 and Fig. 6.42).

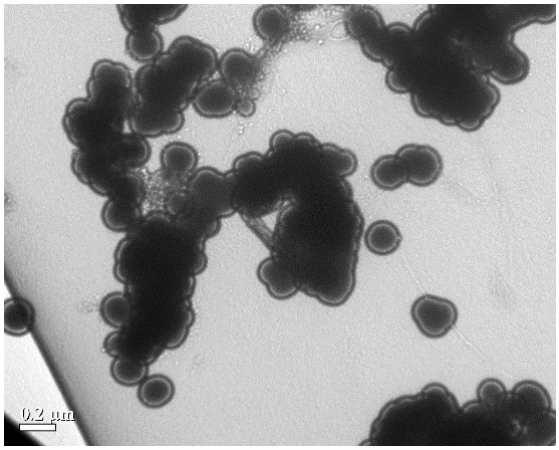

Figure 6.40: Photomicrograph of self-emulsifying formulation of cholecalciferol (SF-2) following dispersion in aqueous media.

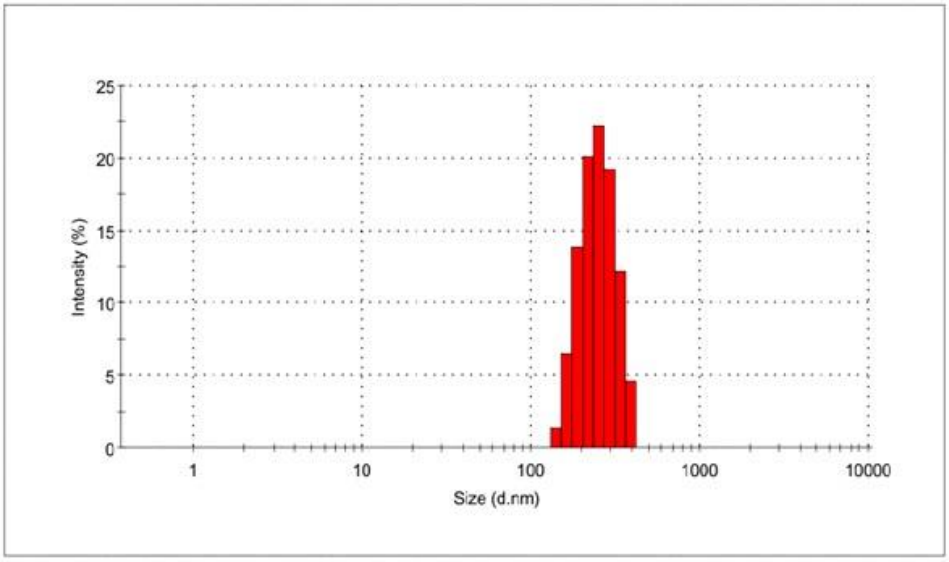

Figure 6.41: Size distribution of self-emulsifying formulation of cholecalciferol (SF-2) following dispersion in aqueous media using dynamic light scattering technique.

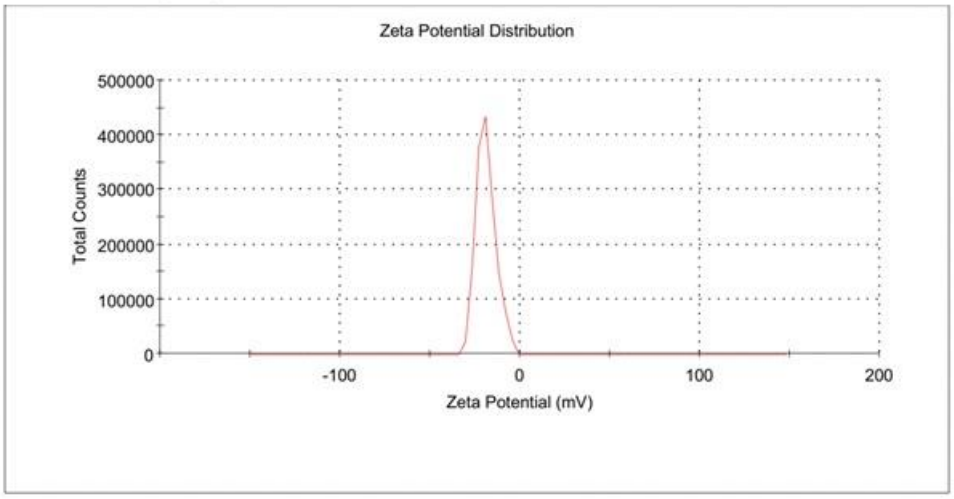

Figure 6.42: Zeta potential of self-emulsifying formulation of cholecalciferol (SF-2) following dispersion in aqueous media.

6.5.3.4 Cholecalciferol content in the SEDDS formulation

The content of the cholecalciferol in the developed formulation (SF-2) was determined to estimate the actual amount of cholecalciferol being entrapped. The formulation exhibited impressive drug content and was found to be 99.21±2.4%.

6.5.4 *In-vitro* stability assessment in SGF and SIF

The effect of pH on formulation stability and robustness to dilution was determined by subjecting SEDDS formulation (SF-2) to 50-, 100- and 1000-fold dilution with simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8). The obtained diluted emulsion was investigated for any physical changes including coalescence of droplets, precipitation or phase separation after 2 h incubation. No coalescence, precipitation or phase separations were seen. The representative images of SF-2 formulation in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8) at 100 times dilution is shown in Fig. 6.43 and Fig. 6.44, respectively.

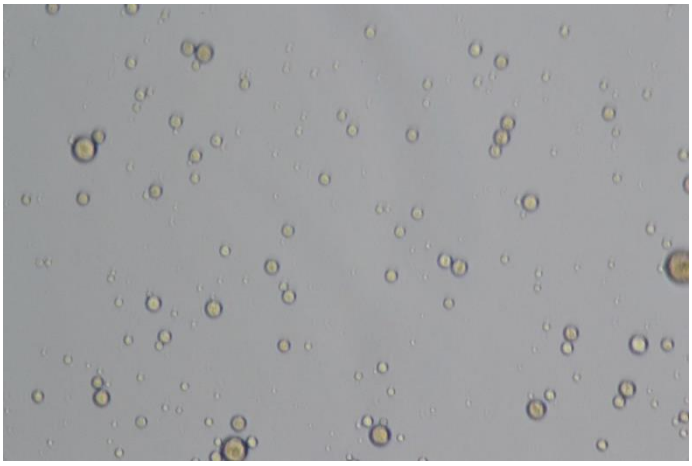

Figure 6.43: Representative photomicrograph of SEDDS formulation (SF-2) in simulated gastric fluid (pH 1.2) at 100 times dilution (40X).

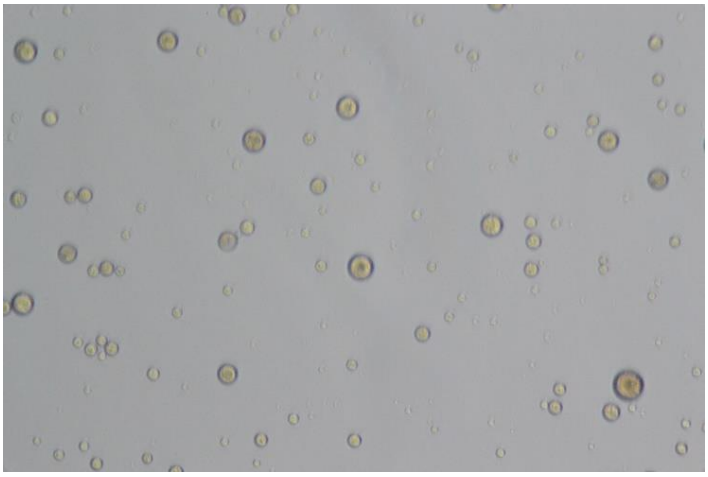

Figure 6.44: Representative photomicrograph of SEDDS formulation (SF-2) in simulated intestinal fluid (pH 6.8) at 100 times dilution (40X).

6.5.5 *In vitro* dissolution study

The dissolution experiment of drugs with low aqueous solubility (such as cholecalciferol) necessitates the inclusion of small amount of surfactant in the dissolution media to provide sink conditions for dissolution. In the gastrointestinal tract, there are natural surfactant such as bile salt which facilitates the absorption of the molecules, therefore the inclusion of surfactant in the dissolution medium is of significance. The dissolution of cholecalciferol from SEDDS formulation (SF-2) was investigated and compared with the dissolution of native crystalline cholecalciferol in simulated gastric medium (pH 1.2). As shown in Fig. 6.45, native crystalline powder of cholecalciferol displayed very low dissolution in order of less than 9 % in period of 2 h. On contrary, SF-2 cholecalciferol SEDD formulation (CCF-SEDDS) showed remarkably high dissolution in order of more than 76% in 2 h. The ability of SEDDS formulation to improve the dissolution of cholecalciferol could be attributed to improve wetting and/or micellar solubilization.

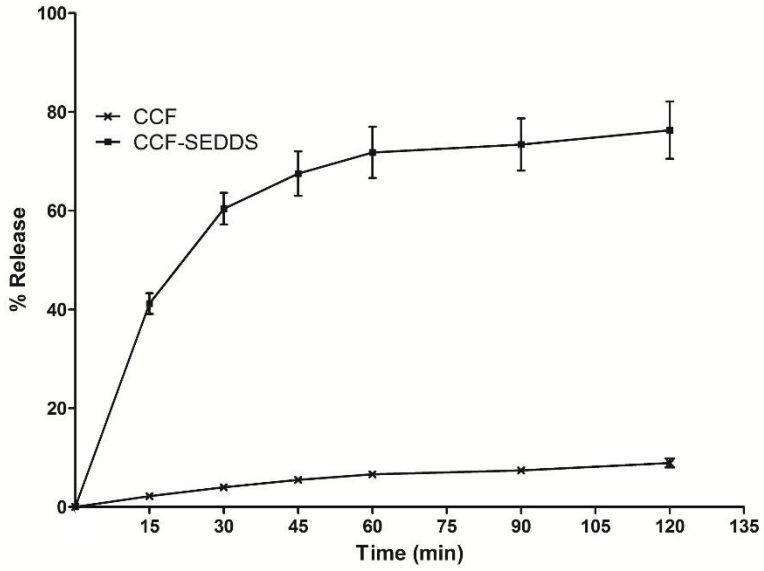

Figure 6.45: Dissolution profile of cholecalciferol SEDDS formulation and crystalline cholecalciferol. The study was performed utilizing a USP II paddle type dissolution apparatus (LABINDIA DS 8000). (P<0.001; CCF-SEDDS Vs CCF).

6.5.6 *In-vitro* bio-accessibility of cholecalciferol

Intestinal absorption of poorly soluble molecule, such as cholecalciferol, from the intestine is rely on their solubilisation in the micro-environment of intestinal through the bile salts mediated emulsification. The hydrophobic molecule can be trapped by the action of bile salt in the mixed micelles which carry that molecule through the intestinal cells barriers and subsequently transport that molecule into the systemic circulation (Porter *et al.,* 2007). It is noteworthy that the bioaccessibility of lipophilic molecule is a prerequisite in order to have bioavailability of that particular molecule. Further, it has already been established that the solubilized material possesses a high potential for absorption by the small intestine (Etcheverry *et al.,* 2012; Fernandez-Garcia *et al.,* 2009). The *in vitro* bioaccessibility of the choleclciferol in SEDDS was evaluated to determine the amount of drug in micelle phase available for absorption. The lipid-based delivery system plays an important role for increasing bioaccessibility especially, SEDDS has the property of easy micellization of the drug molecule thus possesses potential for increased absorption. The bioaccessibility of cholecalciferol using the developed SEDDS formulation was found to be 78.4±5.6%. The observed good bioaccessibility of the choleclciferol could be attributed to its micellar solubilization. One of the constituents of developed formulation is long chain fatty acid which is promptly digested in the intestine. Thereafter lipolysis took place and the resultant digestion moieties are subsequently solubilized by bile salt mixed micelles, which is colloidal in nature with great potential of facilitating absorption (Pouton and Porter 2008). Further, it has been reported that the reduction of the crystallinity is associated with the improved bioaccessibility and bioavailability of molecule (Lindfors *et al.,* 2007). Also, a previous study indicated that the higher bioaccessible amount of

cholecalciferol in nanocomplexes was related with the reduction of the crystallinity of the molecule (Lin *et al.,* 2016).

6.5.7 Effect of formulation on viability of Caco-2 cells

In our study, the effect of various concentrations of cholecalciferol SEDDS formulations (SF-2) on the activity of Caco-2 cells was evaluated by MTT assay (Fig. 6.46). The results are indicating that upon the treatment equivalent to 10, 20, 50 and 100 µg/mL of cholecalciferol in SEDDS, the viability of the Caco-2 cells was more than 85%. It has been demonstrated in the study that the surfactant used in the SEDDS formulation is devoid of any cytotoxic effect on Caco-2 cells and the observations are in accordance to a previous study (Mustafa *et al.,* 2022). Lipid based SEDDS of cholecalciferol in this study comprises oil, surfactant and co-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 (Fischer *et al.,* 2011). However, surfactants are notorious for their local irritation effect, membrane disruption, and cellular death. Therefore, surfactant containing compositions should be assessed for cellular toxicity (Ménard *et al.,* 2012) 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 (El-Sayed *et al.,* 2002; Sandri *et al.,* 2007). We have investigated the effect of formulation on viability of Caco-2 cells and the results showed that the surfactant used in the formulation is devoid of any cytotoxic effect on Caco-2 cells which is in accordance to a previous report (Mustafa *et al.,* 2022).

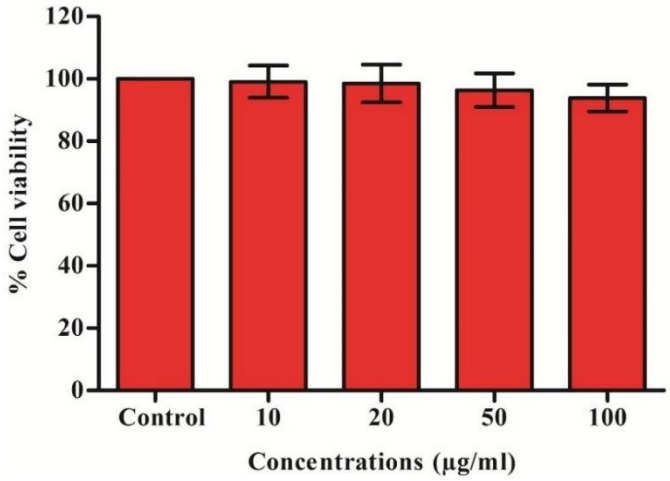

Figure 6.46: Evaluation of biocompatibility of SEDDS with Caco-2 cells by using MTT assay. Caco-2 cells were seeded separately in 96-well flat bottom tissue culture plates at a density of $2x10^4$ cells/well and allowed to grow in monolayer. Once the cells formed monolayer, treatment was given in 96-well plate with 100µL of SEDDS formulation diluted in HBSS solution and kept for 2h incubation. After 2 h treatment, 20µL of MTT solution (2.5 mg/mL) was added in each well and the cells were again incubated for another 4 h to form formazan crystals. The formazon crystals were dissolved by adding 150µL of DMSO using an orbital shaker and absorbance was recorded at 570 nm using a microplate reader (TECAN Infinite Pro) and percent viability was calculated.

6.5.8 Stability study

The stability of the cholecalciferol is influenced by the environmental factors such as light, oxygen, and temperature. Therefore, the stability of the choleclciferol in SEDDS formulation (SF-2) was evaluated by storing the formulations at different storage conditions of temperature and humidity. The formulations SF-2 was 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 cholecalciferol content. The results showed that there was a slight decrease in cholecalciferol content in SEDDS formulation after three months at 30˚C (65% RH). However, there was no significant difference in the cholecalciferol content in SEDDS after three months at 4˚C and 25˚C/60% RH (Fig. 6.47). These findings are in accordance to a previous report which demonstrated

improved the stability of calcitriol with a lipid-based formulation (Yuan *et al.*, 2013). Also, a polymeric formulation demonstrated good stability profile of cholecalciferol at different conditions of storage (Vora *et al.*, 2017). Cholecalciferol is a sensitive micronutrient whose stability is affected by environmental factors. The lipid based SEDDS formulations cause encapsulation of cholecalciferol in lipid carriers leading to decreases in cholecalciferol mobility. Additionally, improved stability may be attributed to the physical barrier for the penetration of oxidizing agents, whose exposure to cholecalciferol is hindered in the SEDDS formulation encapsulated in capsule.

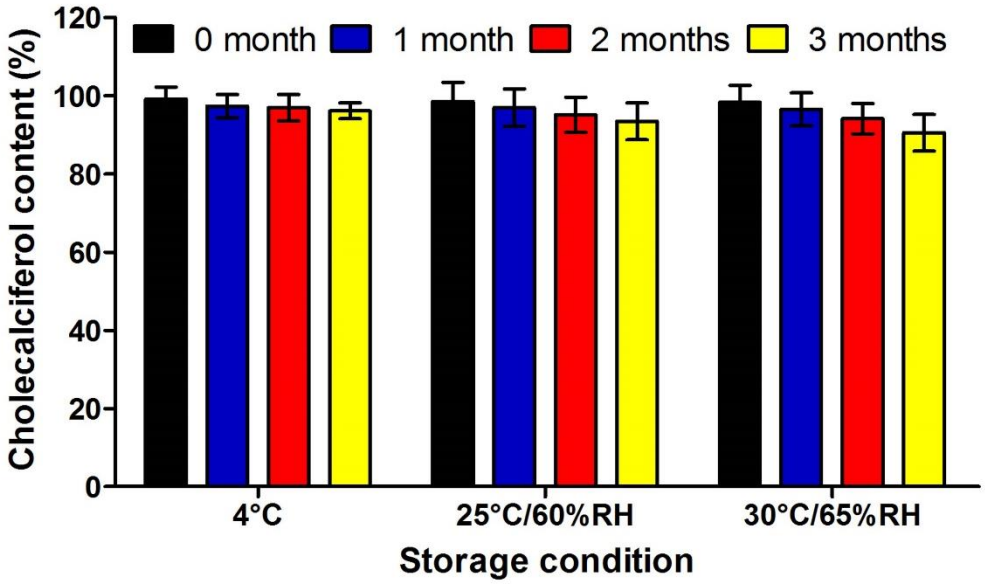

Figure 6.47: Stability studies of cholecalciferol SEDDS. The formulations were placed in sealed containers and stability study was performed by storing the samples at 4˚C (refrigerator), 25˚C (60% RH), and 30˚C (65% RH) for three months. The samples were removed from stability chamber after every month and analyzed for drug content.