International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.1, pp 562-571, Jan-Mar 2010

Influence of method of preparation on solubility, physicochemical properties and in-vitro release profile of Simvastatin- cyclodextrin inclusion complexes: A comparative study

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Abstract :Purpose: This study was performed with the intention of finding the effect of preparation methods on the solubility and dissolution of Simvastatin (SV)-β-cyclodextrin (β-CD) and hydroxypropyl β-cyclodextrin (HPβ-CD) inclusion complexes.
Methods: The complexes were prepared by simple Physical mixing, Kneading and Spray drying techniques. The inclusion complexes were evaluated for phase solubility and in-vitro release study. The complexes were also subjected for physicochemical characterizations.
Results: The differential scanning colorimetry (DSC) and X-ray diffractometry (XRD) results revealed that no endothermic and characteristic diffraction peaks of SV was observed in both the inclusion complexes. The study indicated the conversion crystalline form of SV into the amorphous form. Aqueous solubility and dissolution profiles were markedly increased in inclusion complexes, compared with the drug alone and physical mixture. Moreover, spray dried complexes found better in all the studied parameters compared to the complexes prepared by other methods.
Conclusion: The inclusion complexes prepared by Spray drying method were shown better aqueous solubility and dissolution profile than complexes prepared by other methods.
Key words: Simvastatin, β-CD, HPβ-CD, Spray drying, Inclusion complex, dissolution rate.

Introduction

Simvastatin (SV) is a lipid lowering-agent derived structurally from a fermentation product of Aspergillus terreus [1] and widely used to treat hypercholesterolemia and it is a potent inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonet, which is an early and rate-limiting step in the biosynthesis of cholesterol [2,3]. However, it is practically insoluble in water and poorly absorbed from the gastrointestinal tract [4,5]. Therefore, it is very important to introduce effective methods to enhance the solubility and dissolution rate of drug, substantially leading to its bioavailability.

Cyclodextrins (CDs) are cyclic oligosaccharides, which are produced by enzymatic degradation and have been recognized as useful pharmaceutical excipients [6]. Complexation with cyclodextrins has been reported to enhance the solubility, dissolution rate and bioavailability of poorly water soluble drugs, especially, hydroxypropyl ß-cyclodextrin is widely used in the pharmaceutical field owing to its high aqueous solubility and ability to stabilize the drug molecule [7,8]. The hydrophobic cavity of cyclodextrins in this way increases solubility, bioavailability and stability

[9]. Previously, inclusion complex of SV with α-CD and β-CD was reported [10] but the extensive work is needed. Here, an attempt was made to study the influence of spray drying technique on solubility, physicochemical properties and in-vitro drug release profile, the same were compared with the inclusion complexes prepared by other methods.

Materials and Methods

Simvastatin was received as a gift sample from Lincoln pharmaceuticals Ltd, (Ahmedabad, India). HPβ-CD and β-CD were generous gift received from Gangwal chemicals Pvt. Ltd.(Mumbai, India).All Chemicals and solvents used in the study were of analytical reagent grade. Fresh distilled water was used.

Phase solubility study

50 mg SV was added to 15 ml distilled water containing 0 to10 mM of β-cyclodextrin and transferred to 25 ml stoppered conical flask. The mixture was shaken for 72hrs. Aliquots of 2 ml were withdrawn and filtered immediately using 0.45 µ nylon disc filter. The filtered samples were diluted suitably and assayed for Simvastatin by measuring absorbance at 238 nm against blank. The experiments were conducted in triplicate. The same procedure was followed to HPβ-CD. The apparent solubility constant (K_c) according the hypothesis of 1:1 stoichiometric ratio of complexes was calculated from the phase-solubility diagram using the following equation.

$$K_{a:b} = \frac{\text{slope}}{S_0\,(1\text{-slope})}$$

The slope is obtained from the initial straight line portion of the plot of Simvastatin against cyclodextrin concentration, and S_o is the equilibrium solubility of Simvastatin in water [11].

Preparation of inclusion complexes

Physical mixture

Drug, β-CD and HPβ-CD (CDs) in the molar ratios of 1:1 were mixed separately in a mortar for about one hour with constant trituration, the mixture was passed through sieve # 100 and stored in the desiccators over fused calcium chloride.

Kneading method

SV with β-CD in the molar ratio of 1:1 was taken in a clean glass mortar. First, cyclodertrin was placed in a mortar, a small quantity of 50% methanol was added to it while triturating to get slurry like consistency. Then the drug was slowly incorporated into the slurry and trituration was further continued for one hour. Slurry was then air dried at 25°C for 24 hours, pulverized, passed through sieve # 100 and stored in desiccator over fused calcium chloride. The same procedure was followed for the SV- HPβ-CD.

Spray drying method

The drug and β-CD were dissolved in isopropyl alcohol (IPA) and distilled water separately with the help of a magnetic stirrer. Both the solutions were mixed together on a magnetic stirrer for 30min.The resulting solution was fed to mini spray dryer (Labultima-222,Mumbai,India) and sprayed in the chamber from a nozzle with diameter 0.7mm under the atomization pressure of 1.5kg/cm2 with a feed rate of 3ml/min. The inlet temperature was kept at 80 ˚c and out let temperature 60 ˚c ± 2 ˚c. The vacuum in the system was 60mmwc and aspirator was 45%.The same procedure was adopted to prepare inclusion complex of SV-HPβ-CD. The product thus obtained was collected, packed and doubly wrapped in a aluminum foil and stored in a desiccator till further use [12].

Table 1: Compositions of formulations in molar ratios

Method	Drug to carrier	Drug to carrier ratio	Code
Pure drug Simvastatin			F_0
Physical Mixture	SV:β-CD	1:1	F_1
	SV:HPβ-CD	1:1	F_2
Kneading method	SV:β-CD	1:1	F_3
	SV:HPβ-CD	1:1	F_4
Spray Drying	SV:β-CD	1:1	F_5
	SV:Hβ-CD	1:1	F_6

Evaluation of inclusion complexes

Drug content

Inclusion complexes prepared by physical mixture, kneading, and spray drying methods were assayed for drug content by dissolving a specific amount of the complexes in methanol and analyzed for the drug content spectrophotometrically at 238 nm.

Aqueous solubility

An excess amount of sample was added to 5 ml of the distilled water in test tubes sealed with stoppers. The test tubes were vortex-mixed for 5 min. and then sonicated for 30 min. They were kept in a constant temperature shaking bath maintained at 37 ± 0.5 ˚C until reaching equilibrium (48 h). A portion of the solution was withdrawn and then filtered with a nylon disc filter (0.45 µm) and adequately diluted with methanol [13]. The amount of drug solublised was determined at 238 nm by UV-spectrophotometer (UV-1240, Shimadzu, Japan).

In vitro drug release rate studies

Dissolution study of pure SV and its complexes was performed using USP dissolution apparatus type (USPXX IV). 500 ml of 1.2 pH simulated gastric fluid (SGF) and 7.4 pH Buffer solutions were used as the dissolution media. The study was conducted at 37˚C(± 0.5˚C) with a rotation of 50 rpm. At fixed time intervals 5 ml aliquots were withdrawn, filtered, suitably diluted, and assayed for SV content by measuring the absorbance at 238 nm using a spectrophotometer over a period of two hours. After each sampling, the volume of the dissolution medium was replenished with equal volume of fresh medium at the same temperature to maintain its constant volume throughout the study. The studies were performed in triplicate (n=3). The mean values were calculated for cumulative drug release and the same was used while plotting the release curves. The percent drug released at various time intervals was calculated and plotted against time [14].

Characterization of complexes

X-ray diffraction study (XRD)

The XRD study was done to analyze the powder characteristics of SV and its inclusion complexes. X-ray diffractograms were obtained by Philips diffractometer (PW 1140) and Cu-Kα radiation diffractograms were run at a scanning speed of 2^o/min and a chart speed of 2^o/ 2cm/ 2θ.

Differential scanning calorimetry study (DSC)

The DSC measurements were performed using a Perkin Elmer Pyris (Shelton, CT) and mettler equipped with an intercooler 2P cooling accessory. Samples of 4mg were placed in standard aluminum pans and sealed with a lid. Heating scans by 10^oC/min were applied with a nitrogen purge of 20ml/min, over a temperature range of 30^oC to 285^oC. An empty aluminum pan was used as reference.

Scanning electron microscopy study (SEM)

The morphology of samples was studied using SEM (HITACHI S-3000N,Japan),operated at an accelerating voltage of 20 kV (lament current of 1.75l beam current of 30 – 40 mA and probe current of 250 pA).Samples were prepared by mounting 0.5 mg of powder onto a 5mm silicon wafer a fixed via graphite tape to an aluminum stub. The powder was then sputter-coated for 40 s at beam current of 38 – 42 mA with a 200 A ° layer of gold/palladium alloy.

Particle size analysis

The particle size analysis of both the drug and inclusion complexes was carried out using laser channel beam instrument (CIS-50, Anskermid, Netherland.). The range of particles used for scanning was 1nm -150mm. The lens used was A lens. The particles were suspended in liquid paraffin to give a concentration of 10^{-9} particles /ml with a SNF value of 1. The sample prepared was placed in to the cuvetts made of polystyrene of 1cm path length. The particles were analyzed for their size (length×breadh ×volume) by using laser channel beam.

Results

Phase-solubility study

The apparent solubility constant (K_c) obtained from the slope of the linear phase solubility diagrams was found to be $410.25M^{-1}$ and $727.73M^{-1}$for SV-β-CD and SV-HPβ-CD complexes.

Drug content

The percentage of drug content for all the formulations was found to be between the range of 97.5% and 99.20%.

Aqueous solubility

At the end of 48 hours aqueous solubility of SV was found to be 157µg/ml.Where as, for the physical mixture of SV-HP-βCD and SV-β-CD were found to be 464µg/ml and 399µg/ml respectively. In the formulations prepared by kneading method it was 501µg/ml and 427µg/ml and in spray drying method it was further enhanced to 539µg/ml and 520µg/ml (Table 2).

Figure 1: Phase solubility study of Simvastatin β-CD and HPβ-CD complex.

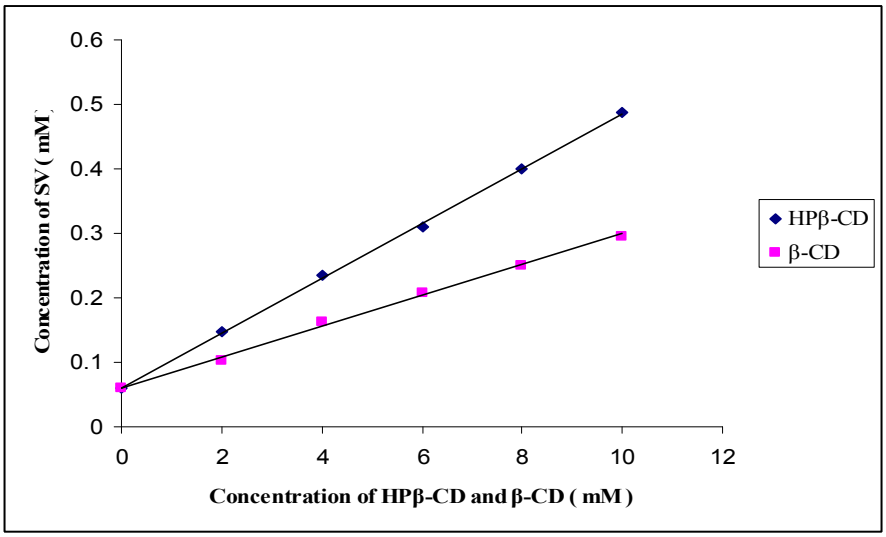


Table 2: Data of Aqueous solubility

SL NO	Formulations	Aqueous solubility in µg/ml
1	F0	157.0
2	F1	399.0
3	F2	464.0
4	F3	427.0
5	F4	501.0
6	F5	520.0
7	F6	585.0

Table 3: Data of Particle size analysis

SL NO	Formulation	Mean Particle size in µm
1	F_0	77.89
2	Formulation F_5	42.80
3	Formulation F_6	33.00

Particle size analysis

The particle size analysis of spray dried pure drug (F_0), formulations F_5 and F_6 was carried out using laser channel beam. The mean particle size of pure spray dried drug is quite bigger than both the spray dried inclusion complexes. However, the particle size of F_5 is little greater than F_6 (Table 3).

Dissolution studies

The percent drug release data from various inclusion complexes was found in the range of 58.68 to 99.60% with in 120 minutes (Table 4&5). The pure drug exhibited only 14 to 35% of release. The formulations F_1, F_3 & F_5 in 1.2 pH buffer solution exhibited the drug release in the order of 65.2%, 88.3%, & 99.5% respectively. Whereas, in the 7.4 phosphate buffer solution it was 68.3%, 89.92%, & 99.50% respectively. The drug release profile of the formulations F_2, F_4, & F_6 in pH 1.2 buffer solution was found to be 65.32%, 99.28% and 99.32% respectively, whereas in 7.4 pH buffer it was 74.82.32%, 99.56% and 99.40% respectively.

Table 4: In- vitro drug release profiles in pH 1.2 Buffer

Time in min	Formulation code						
	F0	F1	F2	F3	F4	F5	F6
30	11.51	35.30	36.40	44.03	74.49	72.82	81.53
60	16.20	48.03	42.34	65.31	86.06	82.20	92.30
90	24.30	55.32	52.52	77.32	94.42	96.30	98.52
120	33.42	65.02	65.32	88.35	99.43	99.52	99.32

Table 5: In vitro drug release profiles in pH 7.4 Buffer

Time in min	Formulation code						
	F0	F1	F2	F3	F4	F5	F6
30	14.20	37.25	40.52	45.08	76.06	74.78	88.25
60	18.32	52.35	50.54	66.06	88.42	89.45	94.30
90	26.62	58.52	65.43	78.52	96.38	96.30	98.25
120	35.42	68.30	74.82	89.92	99.56	99.50	99.40

Figure 2: In vitro drug release study in 7.4 pH Buffer.

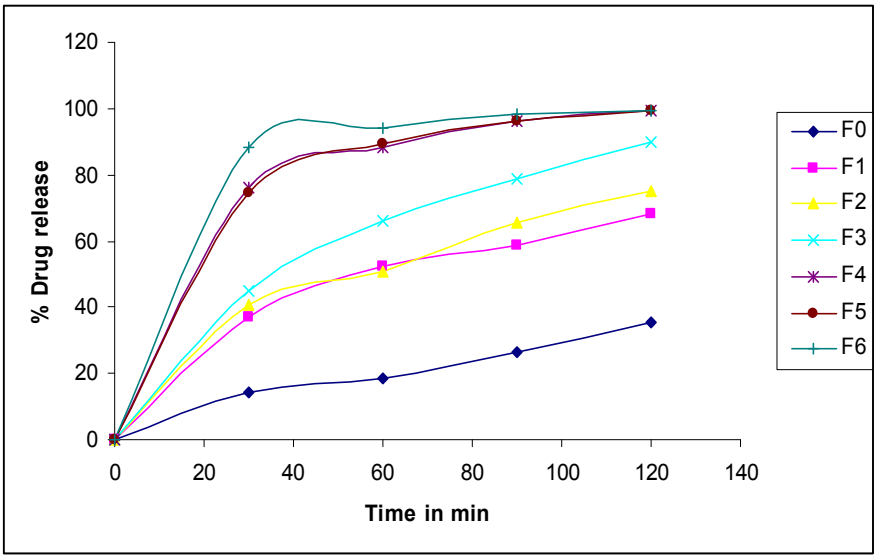
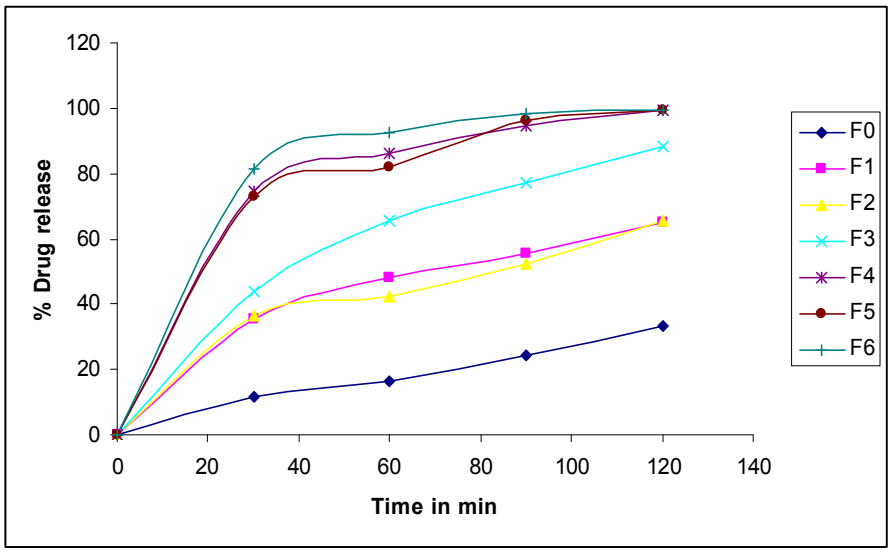


Figure 3: In vitro drug release study in 0.1N HCl.



X-ray diffraction (XRD)

The X-RD patterns of pure drug (SV), β-CD, HPβ-CD, SV:β-CD and SV: HPβ-CD systems are represented in figure 4. The diffractograms of SV and β-CD exhibited a series of intense peaks, which is an indicative of their crystalline nature. X-RD pattern of physical mixture F_1 and F_2 is simply the superimposition of each component indicating no formation of new structure. Complex prepared by kneading method F_3 and F_4 showed a diffraction pattern quite similar to that of physical mixture, while those obtained from Spray drying method F_5 and F_6 showed less peaks with low intensity.

Differential scanning calorimetry (DSC)

The DSC thermograms of pure SV, β-CD and HPβ-CD and corresponding cyclodextrin complexes are presented in figure 5. The DSC thermogram of F_0 (SV) showed an endothermic peak at 138.17°C corresponding to its melting point. The thermograms of SV-β-CD F_1 showed endothermic peaks at 97.23°C and F_3 showed 130.15°C This may be due to shift of characteristic peak of SV which was observed at 138.17°C, indicates a strong interaction of drug and β-CD. In case of SV and β-CD complexes (1:1 M) prepared by spray drying method exhibited a broad endothermic peak at 161.2°C instead of 138.17°C indicating strong interaction between drug and β-CD at 1:1 molar ratio. The thermograms of SV and HPβ-CD (1:1 M) prepared by physical mixture and kneading method (F_2 and F_4) respectively showed endothermic peaks at 102-154°C. This may be due to shift of characteristic peak of SV which was observed at 138.17°C, indicates a strong interaction of drug and HPβ-CD.

Scanning electron microscopy (SEM)

SEM is used to asses the microscopic surface morphology of the drug and complexes. Pure drug is characterized by the presence of crystalline particle of a regular size. The physical mixture of SV-β-CD and SV- HPβ-CD showed slight crystalline structure of both drug and complexing agents. Crystals of drug mixed with crystals of complexing agents were seen adhering to there surface. The SEM photographs of inclusion complexes prepared by kneading method seen to be slightly amorphous structure for both drug and complxing agents. The photographs of spray dried inclusion complexes showed the characteristic morphology as a small sized particles tending to aggregation, indicating the existence of an amorphous product with presence of single component in the complex thus suggesting maximum complexation.

Figure 5: XRD spectrum of formulations (A) HPβ-CD (B) β-CD (C) F_0-formulation (D) F_1-formulation (E) F_2-formulation (F) F_3-formulation (G) F_4-formulation (H) F_5-formulation (I) F_6–formulation.

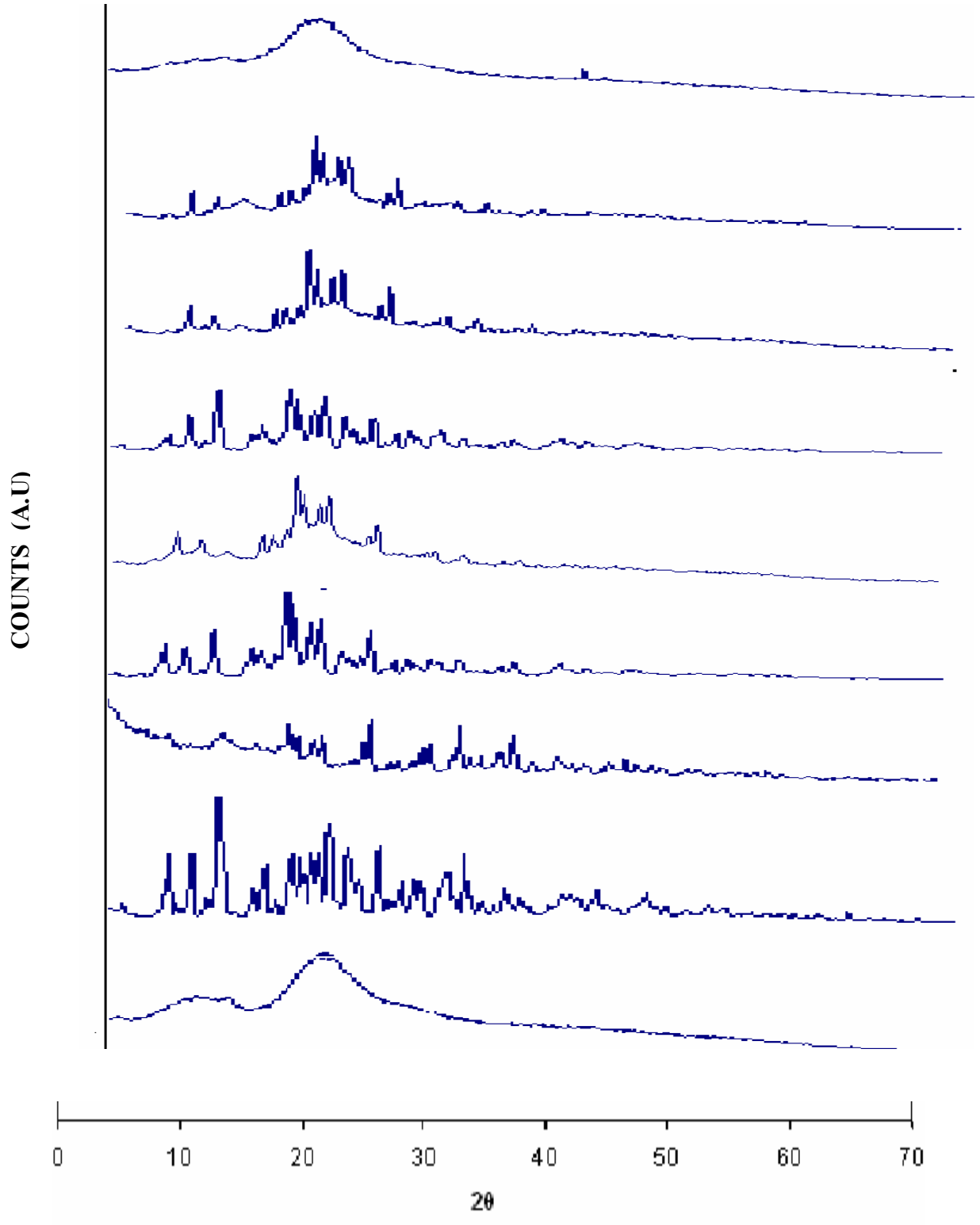


Figure 6: DSC Thermograms of formulations (A) HPβ-CD (B) β-CD (C) F_0 -formulation (D) F_1 -formulation (E) F_2 -formulation (F) F_3 -formulation (G) F_4 -formulation (H) F_5 -formulation (I) F_6 –formulation.

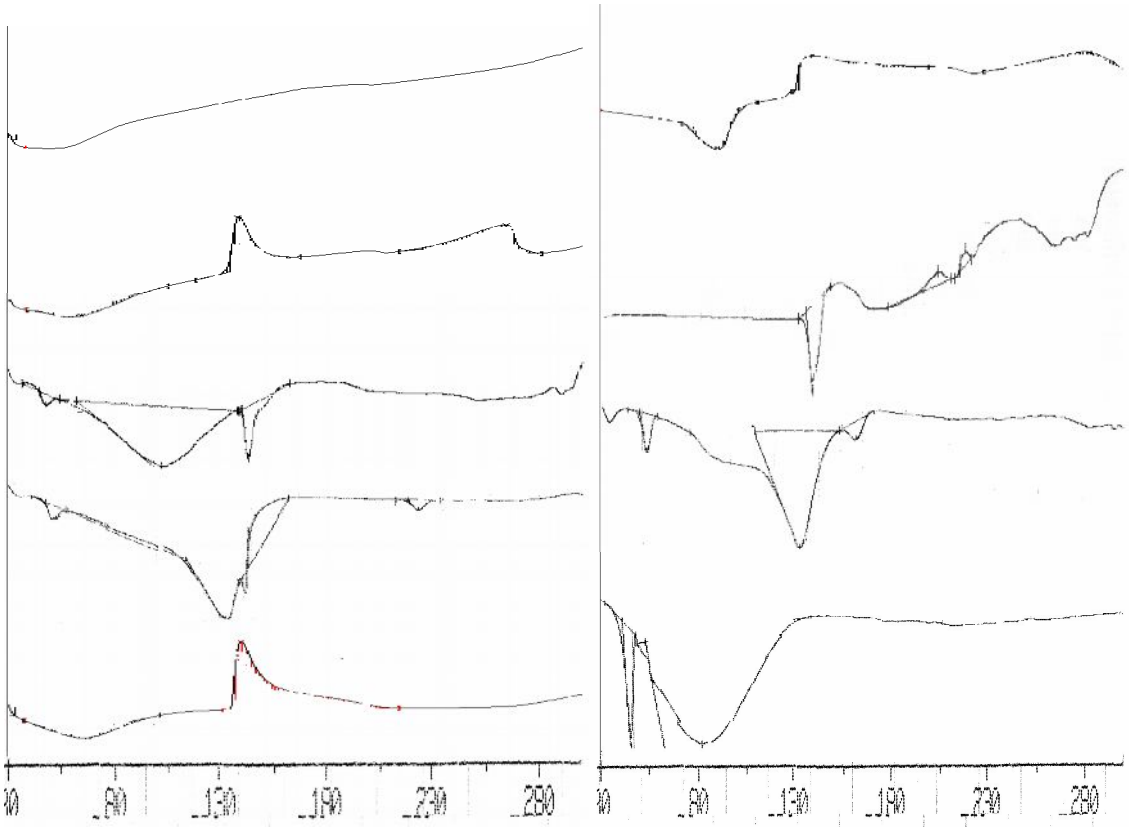
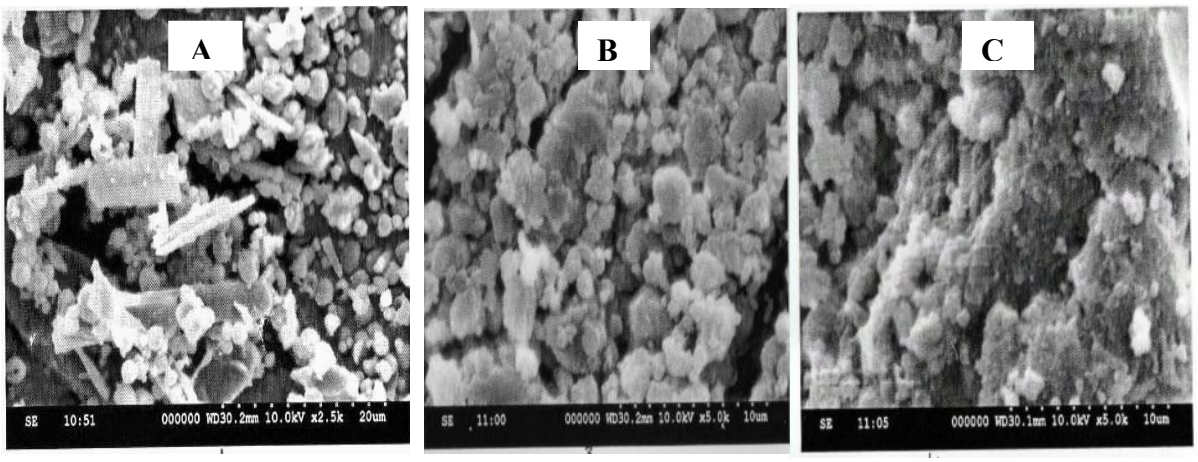
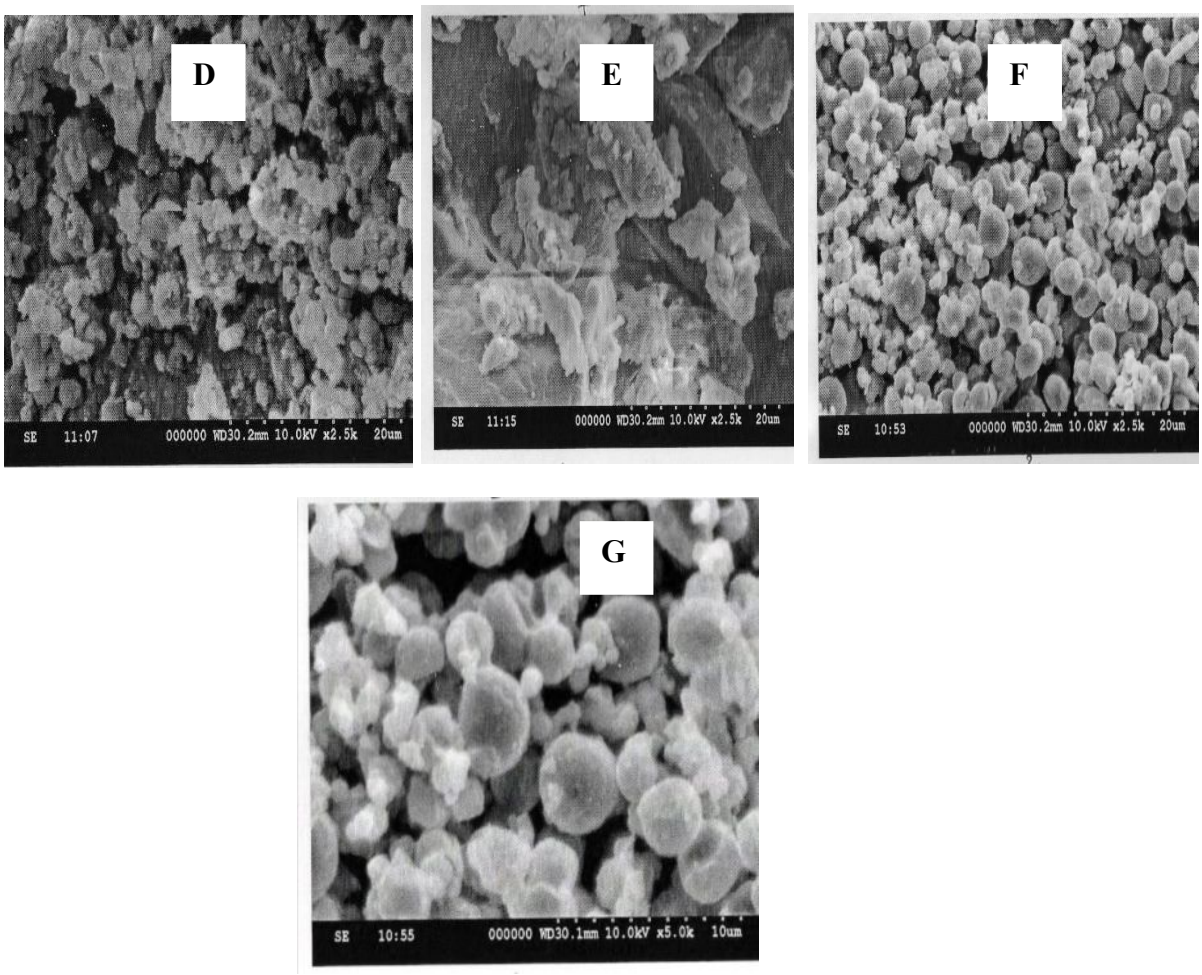


Figure 7: SEM of formulations (A) F_0 -formulation (B) F_1 -formulation (C) F_2 -formulation (D) F_3 -formulation (E) F_4 -formulation (F) F_5 -formulation (G) F_6 –formulation.





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