

PREPARATION & *IN-VITRO* EVALUATION OF INCLUSION COMPLEXES OF SIMVASTATIN TABLET WITH CYCLODEXTRINS

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ABSTRACT

In the present study, the practically insoluble drug simvastatin (SV) and its inclusion complex with hydroxypropyl β -cyclodextrin (HP- β -CD) prepared using different process were investigated to improve the aqueous solubility and the dissolution rate of drug, thus enhancing its bioavailability. Inclusion complexation in aqueous solution and solid state was evaluated by the phase solubility diagram, differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD), Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). The phase solubility diagram with HP- β -CD was classified as type A indicating the formation of 1:1 stoichiometric inclusion complex. The apparent complexation constants ($K_{1:1}$) calculated from phase solubility diagram were 103 and 105 Mat^{-1} 25°C . No characteristic diffraction peaks corresponding

to SV was observed for the inclusion complex in PXRD. Complex shows resulting in the formation of amorphous form. Aqueous solubility and dissolution studies indicated that the dissolution rates were remarkably increased in inclusion complex, compared with the physical mixture, Kneading and drug alone. This could be primarily attributed to the improved solubility and dissolution associated with inclusion complex between drug and HP- β -CD. In conclusion, spray drying process could be a useful method for the preparation of the inclusion complex of drug with HP- β -CD and its solubility, dissolution rate and hypolipidemic activity were significantly increased by complexation between SV and HP- β -CD.

KEYWORDS: Simvastatin; Hydroxypropyl- β -cyclodextrin; Inclusion complex; Spray

drying process.

INTRODUCTION

Simvastatin (SV) is a cholesterol-lowering agent that is derived synthetically from a fermentation product of *Aspergillus terreus*^[1] and widely used to treat hypercholesterolemia. Simvastatin is in reality a prodrug with an inactive lactone, which is metabolized in the liver to its active form (the corresponding β -hydroxyacid),^[2] as shown here in Figure 1.^[2]

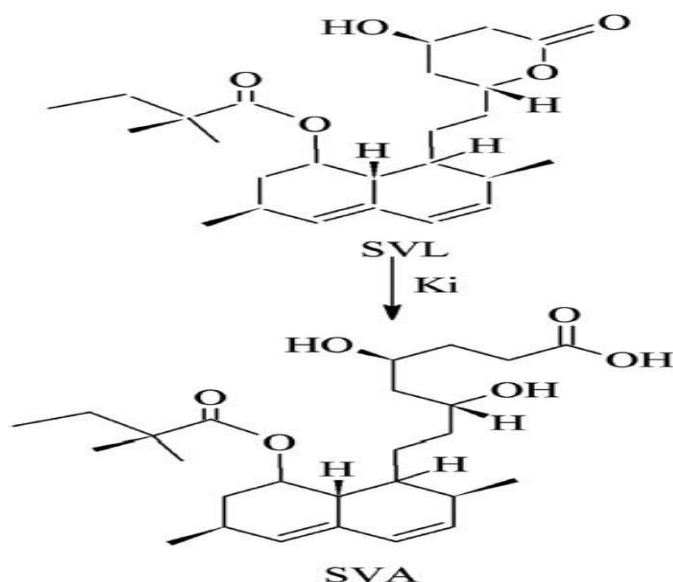


Figure 1: Simvastatine lactone prodrug (SVL) conversion to from active β -hydroxy acid (SVA).^[2]

SV is a potent inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMGCoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol.^[3,4] Simvastatin, a well known statin with antilipemic activity, can be classified as a Class II drug (BCS).^[5] It is practically insoluble in water and poorly absorbed from the gastrointestinal (GI) tract.^[6,7] It is very important to introduce methods to enhance the solubility and dissolution rate of drug, substantially increase to its bioavailability.

Enzymatic hydrolysis of starch usually results in formation of glucose, maltose and a long range of linear and branched dextrans. However, a number of different microorganisms and plants produce certain enzymes called cyclodextrin glucosyltransferases (CGTs), which degrade starch to cyclic products called cyclodextrins. These are cyclic oligosaccharides consisting of a lipophilic central cavity and a hydrophilic outer surface. Because of such

characteristics, cyclodextrins form inclusion complexes both in solution and in solid state, in which each guest molecule is surrounded by the hydrophobic environment of the cyclodextrin cavity. This can lead to alteration of the physical, chemical and biological properties of the guest molecules and can eventually have considerable pharmaceutical potential.^[8] Owing to complexing ability, they have found extensive application in many fields including pharmaceutical technology (to improve the aqueous solubility, dissolution rate, bioavailability and stability of drugs.^[9,11] Now, many poorly soluble drugs have been complexed by cyclodextrin to enhance solubility, chemical stability and bioavailability of the drugs.^[12,16] Out of the three parent cyclodextrins, β -cyclodextrin (β -CD) appears most useful as a pharmaceutical complexing agent because of its complexing ability, low cost and other properties. There are two types of complex cyclodextrin forms with the guest molecules, type A (L) that forms in solutions and type B (S) which forms in solid complex.^[17] Apart from the kneading^[18], the solid drug can be complexed with β -CD by freeze drying^[19], spray drying^[20], co-evaporation^[21] or by roll mixing.^[22] Currently, there are over 30 marketed pharmaceutical products based on cyclodextrin complexes.^[23] Therefore, Cyclodextrin was selected to form an inclusion complex with Simvastatin to enhance its solubility. The major limitation of Simvastatin is poor solubility and dissolution rate. Hence different methods are applied to preparation of inclusion complex. The type of complexation and complexation constant evaluated from phase solubility study. The solid properties of the inclusion complex were characterized by the Powder X-ray diffractometry (PXRD), Differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT- IR) and Scanning electron microscopy (SEM).

2. MATERIALS AND METHODS

2.1 MATERIALS

Simvastatin as a drug gift sample from Alkem pharmaceutical and Cyclodextrin was obtained from Rajesh chemicals, Mumbai. All other chemicals were analytical grade and used further purification. The instruments used are eight station punching machine (chamunda), eight stage tablet dissolution apparatus (Electrolab, Mumbai), UV Spectrophotometer (Schimadzu, Japan), Fourier Transform Infrared spectrophotometer (FTIR) (Bruker), Differential Scanning Calorimetry (DSC) (Shimadzu), Field Emission Scanning Electron Microscopy (FESEM) (NOVA NANOSEM 450).

2.2 PHASE SOLUBLITY DIAGRAM

Solubility studies were performed according to the method reported by Higuchi and Connors.^[24] Excess simvastatin was added to purified water (pH 6.8) containing various concentrations of HP- β -CD (0.002–0.01 M) in a series of 100 ml volumetric flasks and the mixture was shaken for 48 h at room temperature (25°C) on a shaker (120 rev./min). Then, the samples were kept aside to achieve equilibrium. After equilibrium was reached aliquots were then filtered through Whatman filter paper. The filtered samples were diluted suitably and assayed for simvastatin, by measuring the absorbance at 240 nm. The phase solubility diagram was plotted total drug concentration against concentration of HP- β -CD. The apparent complexation constant ($K_{1:1}$) of the complex was calculated as following equation (Eq. (1) from phase solubility slope, where the intercept is the intrinsic solubility of drug in the absence of HP- β -CD at 25°C;

(1)

$$K_{1:1} = \frac{\text{Slope}}{\text{Intercept} (1 - \text{Slope})}$$

2.3 PREPARATION OF INCLUSION COMPLEX 1] Physical mixture

The physical mixture (1:1 molar ratio) was prepared by mixing of pulverized powder of drug and cyclodextrin. The material was passed through sieve (#85).

2] Kneading method

Thick slurry was prepared by adding one third water by weight to cyclodextrin. Under stirring the quantity of drug (1:1 molar ratio) was added to it and then dried in an oven at 40°C until dry. The dried mass was pulverized and sieve through mesh (#85).

3] Spray drying

The simvastatin dissolved in the methanol and cyclodextrin in the water (1:1 molar ratio). The two solution was mixed slowly with stirring until uniformly mix. The solution was subjected to spray drying (Labultima LU-222 Advanced).

2.4 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

DSC determination of samples were conducted on Shimadzu DSC 60 (Shimadzu corporation, Japan). Temperature and enthalpy were calibrated with the standard materials indium (melting point = 156.6°C) and zinc (melting point = 419.5°C) at a heating rate of 5°C/min. The accurately weighed samples (1 mg) sealed in the aluminum pans and the heated

at rate of 10°C/min. The measurements were performed at heating range of 20-300°C under a nitrogen purge gas flow rate of 40ml/min was used for DSC run.

2.5 POWDER X-RAY DIFFRACTOMETRY (PXRD)

Powder X-ray diffraction patterns of samples were obtained using powder X-ray diffractometer (Generator: Bruker axs D8 advance), with Nickel 40 K α as the source of radiation, which was operated at the voltage 40 kV and the current 45 mA. Each sample was placed in the cavity of an aluminum sample holder flattened with a glass slide to present a good surface texture and inserted into the sample holder. In order to measure the powder pattern, the sample holder and detector were moved in a coupled mode path to determine the angles of scattered radiation and to reduce preferred sample orientation. All samples were measured in the 2 θ angle range between 5° and 60° with a scan rate of 3°/min and a step size of 0.1°. All samples were analyzed in duplicate.

2.6 FOURIER-TRANSFORM INFRARED (FT-IR) SPECTROSCOPY

Fourier-transform infrared spectra were recorded on Shimadzu FT-IR-4800 (Shimadzu corporation, Japan). The FT-IR measurements were performed at frequency range of 4000-400 cm⁻¹ at ambient temperature. The resolution were used 4 cm⁻¹ and 96 scans were co-added for all samples.

2.7 FILED EMISSION SCANNING ELECTRON MICROSCOPY (FESEM)

Filed emission scanning electron microscopy was used to determine the morphology of Simvastatin, HP- β -CD and simvastatin/HP- β -CD inclusion complex by physical mixture, kneading method, spray drying. The morphology of these samples was determined using the field emission scanning electron microscope (FESEM) FEI, NOVA NANOSEM 450. The pictures were taken at excitation voltage of 5 kv and magnification of 5000, 10000x. Samples were prepared by mounting approximately 0.5 mg of powder on to a silicon wafer via carbon tape to an aluminum stub. The powder was sputter-coated for 120 s at beam current of 20 mA with a 200 Å layer of chromium.

2.8 DRUG CONTENT

Simvastatin inclusion complex with HP- β -CD by physical mixture, Kneading method and spray dried were taken equivalent to the 10 mg of the simvastatin was weighed and dissolved in suitable quantity of methanol. The drug content was determined at 240 nm by UV – spectrophotometer (Jasco-V-630).

2.9 AQUEOUS SOLUBILITY

An excess amount of sample was added to 5 ml of the phosphate buffer solution (PH 6.8) in 10 ml volumetric flask and sealed with stoppers. The volumetric flask were vortex-mixed for 15 min and then sonicated for 60 min. Then volumetric flask were kept in a constant temperature orbital shaking incubator maintained at $37 \pm 0.5^\circ\text{C}$ until reaching equilibrium for 48 Hrs. A portion of solution was withdrawn and filtered with whatman filter paper. The solution was adequately diluted with methanol. The drug concentration determined at 240 nm by UV- spectrophotometer (Jasco-V-630).

2.10 DISSOLUTION STUDY

The in-vitro dissolution studies of pure drug, physical mixture, Kneading method and Spray dried complexes were carried out in USP type II dissolution test apparatus (Electrolab Ltd. TDT-08L) with paddle stirrer. In the studies, the samples equivalent to 10 mg of simvastatin was placed in the dissolution vessel containing 900 ml phosphate buffer (pH 6.8) maintained at $37 \pm 0.5^\circ\text{C}$ and stirred at 100 rpm. Samples from dissolution media have withdrawn at different time intervals and filtered with whatman filter paper, replaced with a fresh dissolution medium.

Concentration of simvastatin was determined by using spectrophotometric method by measuring absorbance at 240 nm (Jasco-V-630).

3. RESULTS AND DISCUSSION

3.1 THE PHASE SOLUBILITY DIAGRAM

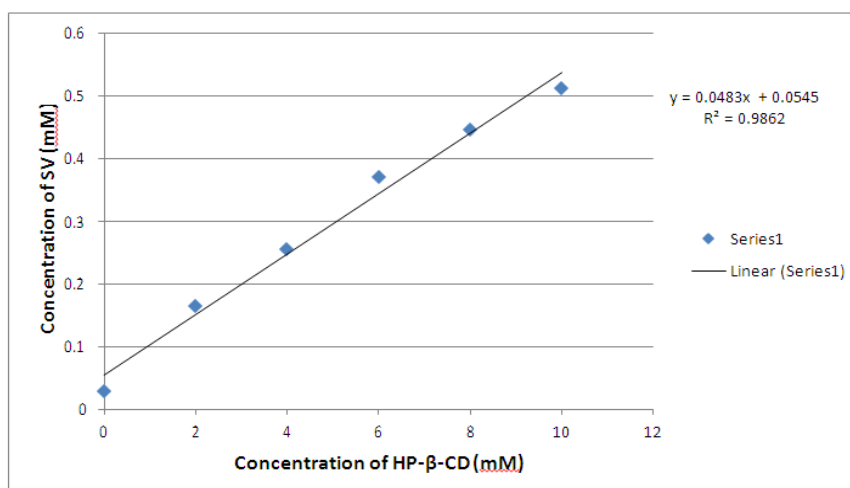


Fig. No. 1: Phase solubility diagram of SV as function of HP-β-CD Concentration at temperature of 25°C.

The phase solubility diagram for the complex formation between simvastatin and HP- β -CD is shown in Fig. No. 1. The aqueous solubility of simvastatin increased linearly with a slope 0.0483 ($r = 0.9862$) as a function of HP- β -CD concentration. The phase solubility diagram (Fig. 1) can be classified as type (A) according to Higuchi and Connors.^[24] It is assumed that the increase in solubility observed was due to the formation of a 1:1 M inclusion complex between simvastatin and HP- β -CD. The stability constant ($K_{1:1}$) of simvastatin and HP- β -CD complex (1:1) was calculated as 105 M^{-1} . The UV spectra of solution in the presence of increasing molar concentration of HP- β -CD were recorded. The observed changes in peak intensity are assumed to result from changes in the solvent microenvironment upon inclusion of the solute.

3.2 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Differential scanning calorimetry (DSC) diagram of simvastatin, HP- β -CD, inclusion complex of simvastatin/HP- β -CD by physical mixture, kneading method and spray drying are presented in fig. no. 2.1 to 2.5.

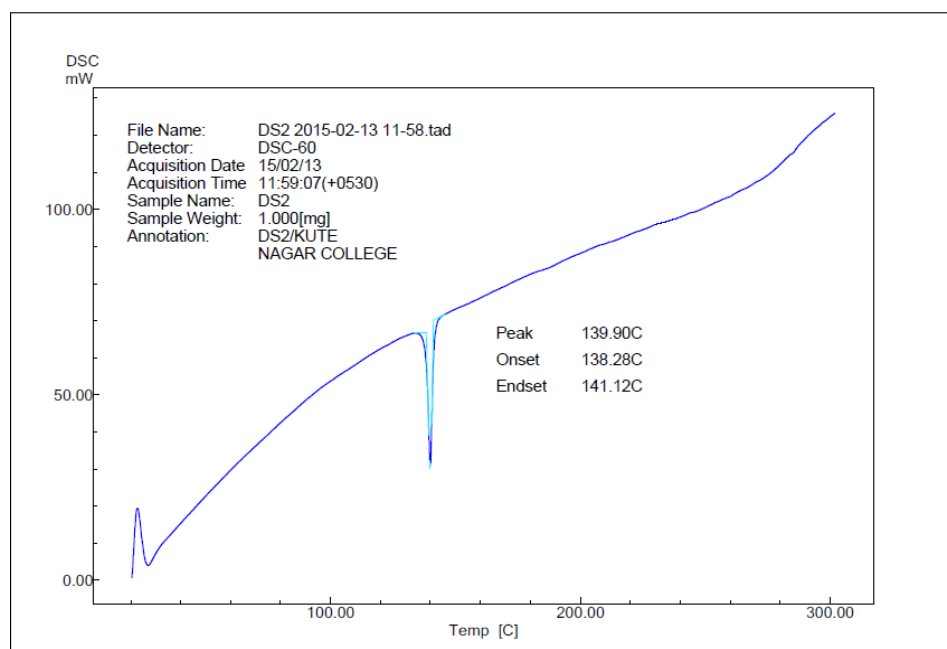


Fig. No. 2.1: DSC thermogram of simvastatin.

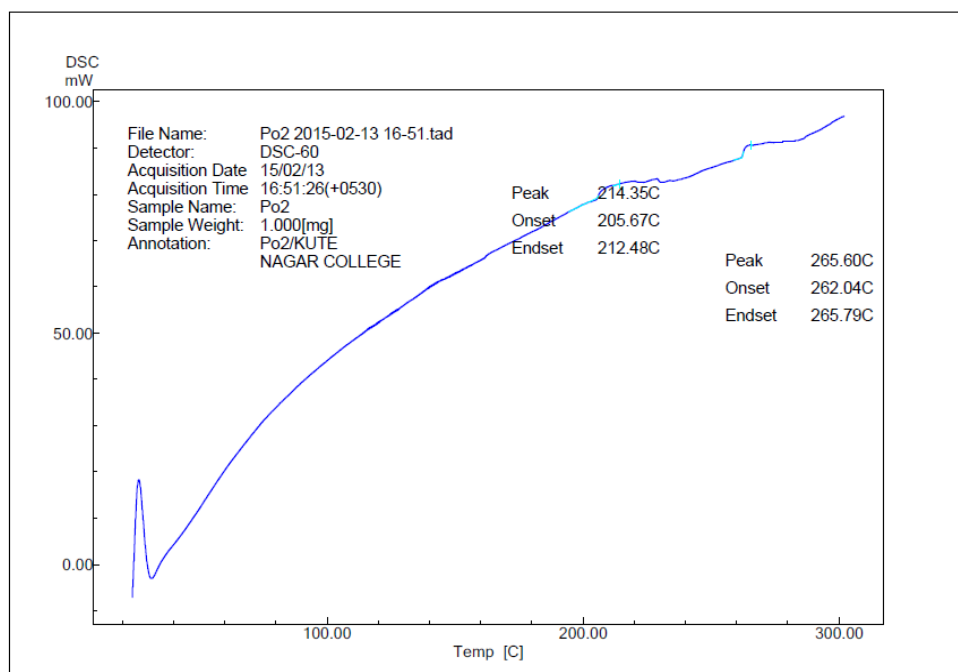


Fig. No. 2.2: DSC thermogram of HP-β-CD.

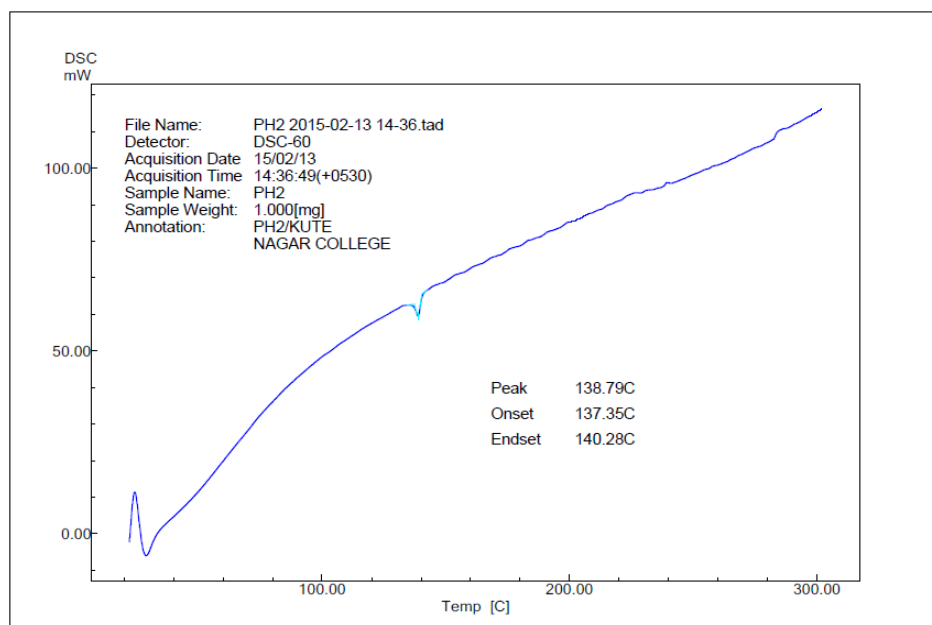


Fig. No. 2.3: DSC thermogram of simvastatin/HP-β-CD by physical mixture.

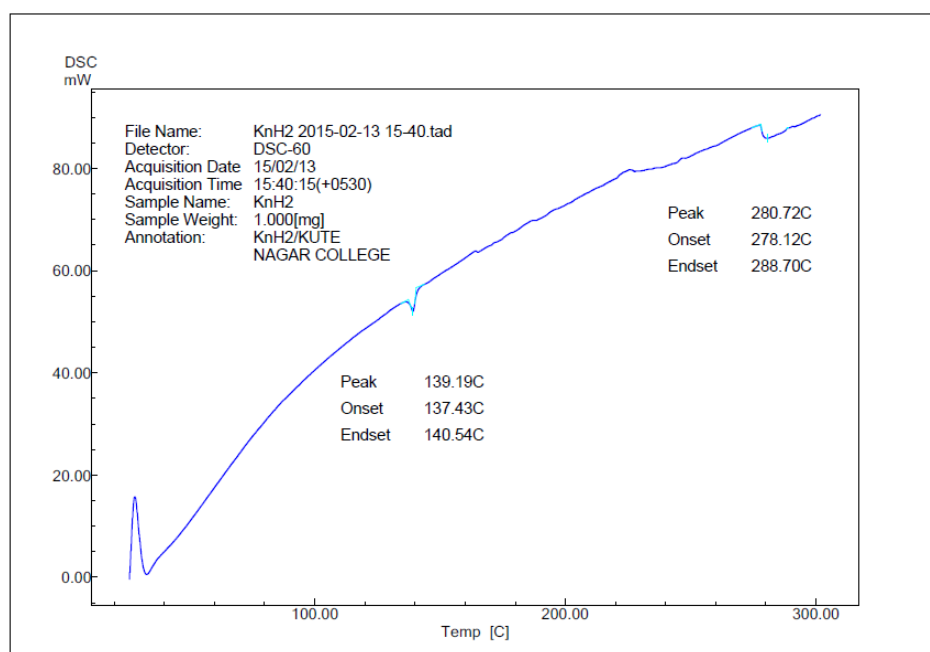


Fig. No. 2.4: DSC thermogram of simvastatin/HP-β-CD by kneading method.

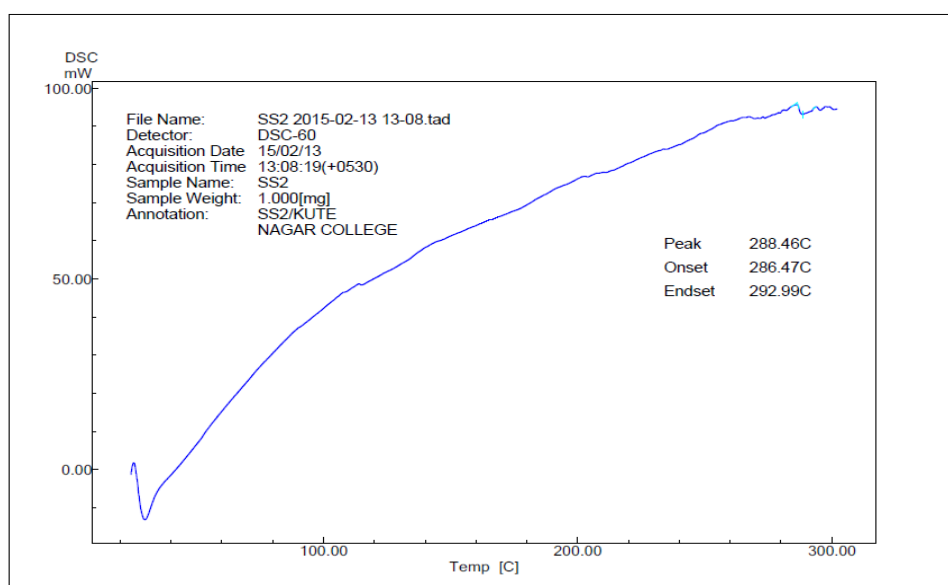


Fig. No. 2.5: DSC thermogram of simvastatin/ HP-β-CD by spray drying.

The DSC thermogram of simvastatin exhibited single, sharp endothermic peak at 139.90°C (fig. no. 2.1), corresponding to its melting point. The thermogram of HP-β-CD showing peak at 214.35°C and 265.60°C (fig. no. 2.2). In the DSC curves of simvastatin/HP-β-CD inclusion complex by the physical mixture showing narrow endothermic peak at 138.79°C, indicating the presence of the trace amount of simvastatin (fig. no. 2.3). The DSC curve of simvastatin/HP-β-CD by kneading method shows two peaks endothermic melting peak at 139.19 characteristic of simvastatin and other peak at the 280.17 (fig. no. 2.4). In contrast the

thermogram of inclusion complex of simvastatin/HP- β -CD by spray drying does not consist any endothermic peak at coresponding to melting point of simvastatin (fig. no. 2.5), indicating the formation of amorphous inclusion complex. Showing the molecular encapsulation of the drug inside the HP- β -CD cavity.

3.3 POWDER X-RAY DIFFRACTOMETRY (PXRD)

Powder X-ray diffractometry is a useful method for the detection of cyclodextrin complexation in powder or microcrystalline states. The powder X-ray diffraction patterns of simvastatin, HP- β -CD, simvastatin/HP- β -CD inclusion complex by physical mixture, kneading method and spray drying are represented in Fig. no. 3.1 to 3.5.

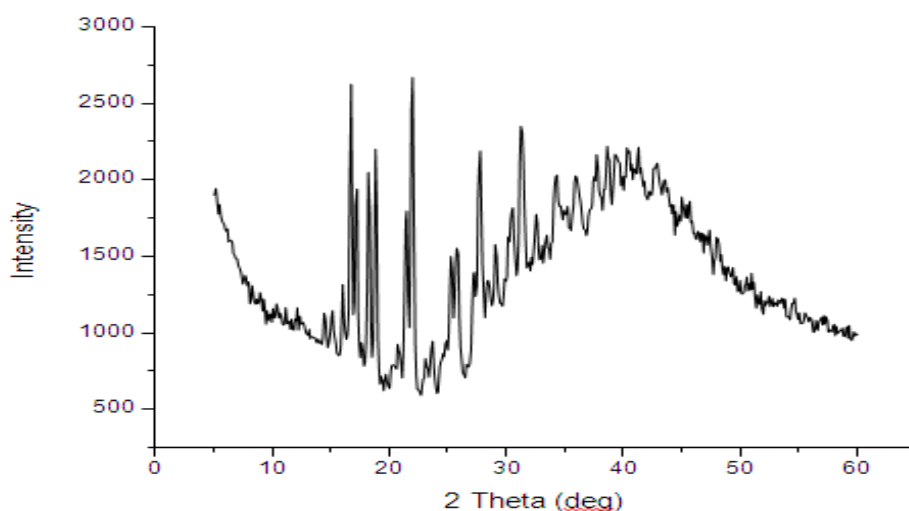


Fig. No. 3.1: Powder X-ray diffraction pattern of simvastatin.

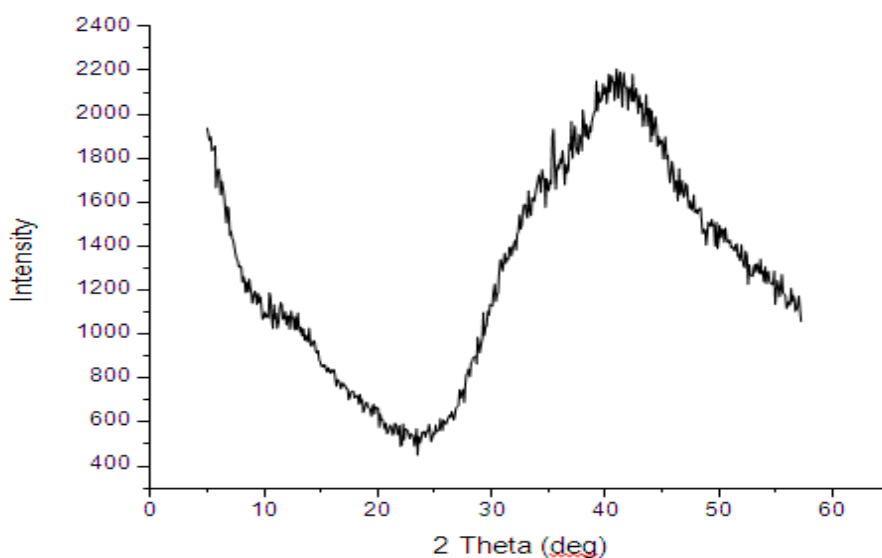


Fig. No. 3.2: Powder X-ray diffraction pattern of HP- β -CD.

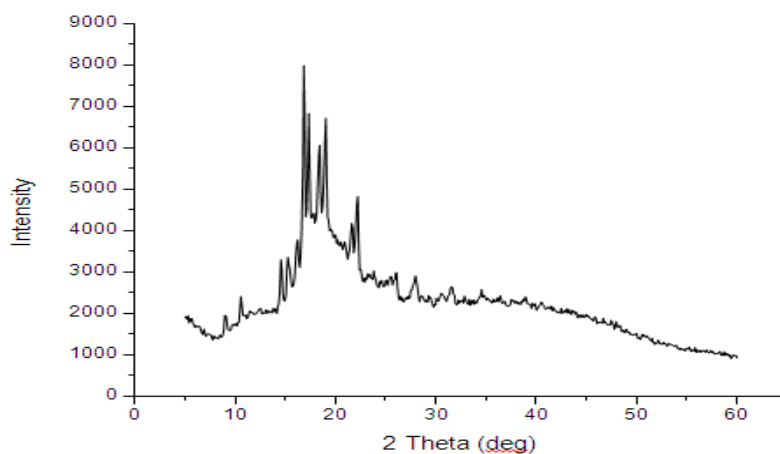


Fig. No. 3.3: Powder X-ray diffraction pattern of simvastatin/HP-β-CD complex by physical mixture.

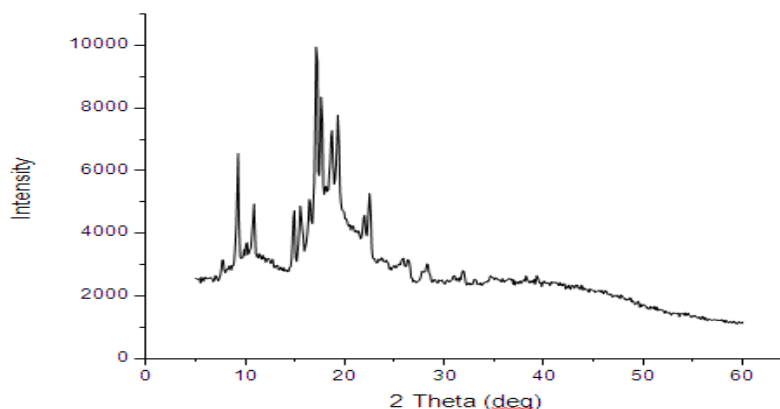


Fig. No. 3.4: Powder X-ray diffraction pattern of simvastatin/HP-β-CD complex by kneading method.

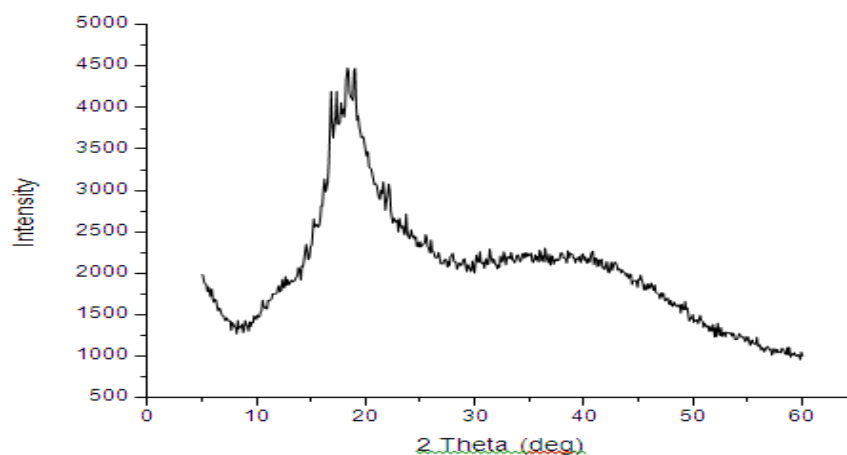


Fig. No. 3.5: Powder X-ray diffraction pattern of simvastatin/HP-β-CD complex by spray drying.

The powder diffraction pattern of simvastatin several sharp peaks at diffraction angles of 2θ 16.6, 17.4, 18.6, 19.2, 22.5, 22.4, 28.3 and 31.9 suggesting that the drug is present in crystalline form (fig. no. 3.1). However HP- β -CD was lacking the peaks shows as an amorphous form (fig. no. 3.2). The several peaks present in simvastatin/HP- β -CD inclusion complex by physical method (fig. no. 3.3) and kneading method (fig. no. 3.4) indicating the simvastatin maintain its crystallinity. The diffraction pattern of simvastatin/HP- β -CD complex by the spray drying method was similar to that of the amorphous HP- β -CD and the several sharp crystalline peaks of simvastatin was disappeared (fig. no. 3.5). These results indicate the drug does not present in the crystalline form when inclusion complex with the HP- β -CD by the spray drying method. Disappearance of peaks indicates some new complex compounds were formed and existed in amorphous state. These gives the supporting evidence for the formation inclusion complex between simvastatin and HP- β -CD.

3.4 FOURIER-TRANSFORM INFRARED (FT-IR) SPECTROSCOPY

The FT-IR spectra of simvastatin, HP- β -CD, simvastatin/HP- β -CD inclusion complex by physical method, kneading method and spray drying represented in Fig. no. 4.1 to 4.5.

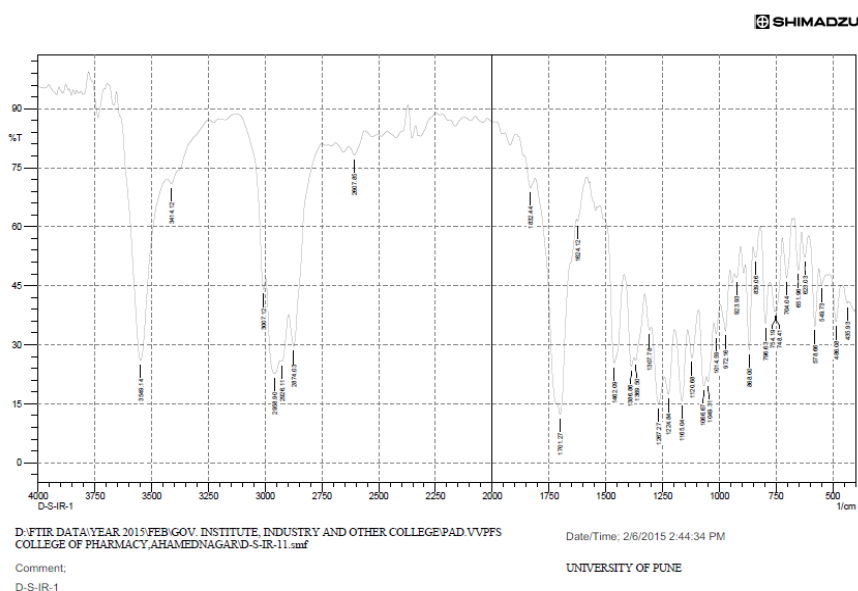


Fig. No. 4.1: FT-IR spectra of simvastatin.

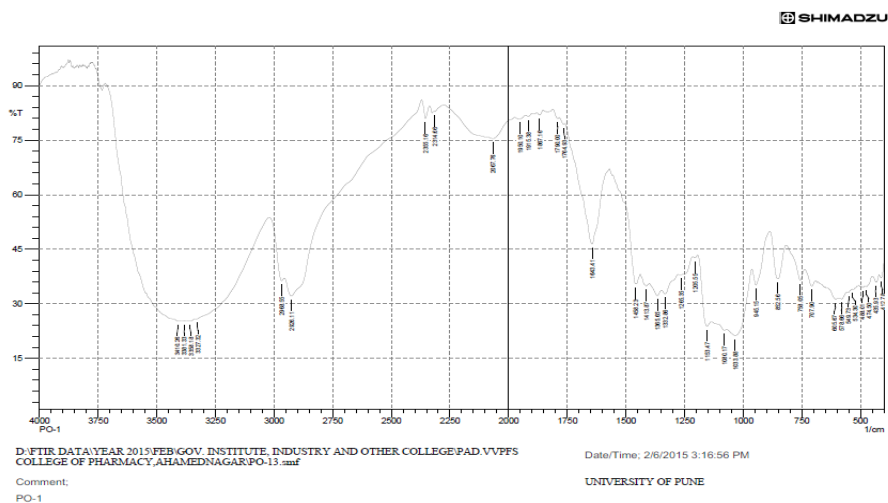


Fig. No. 4.2: FT-IR spectra of HP-β-CD.

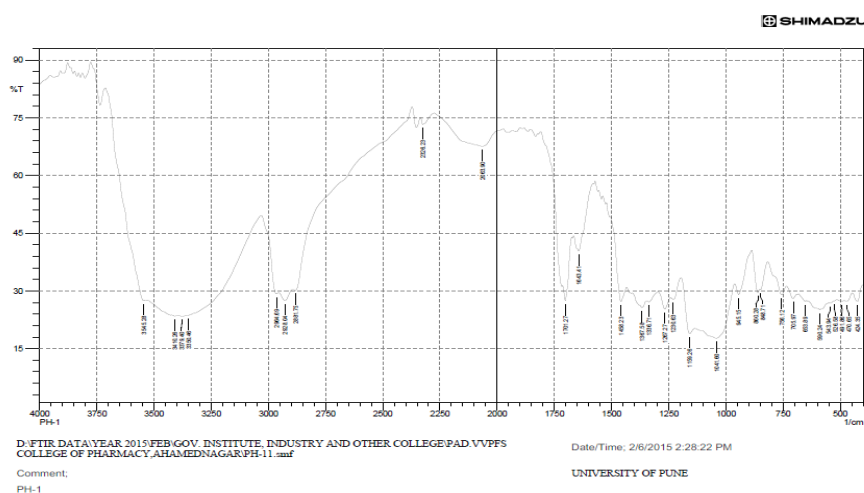


Fig. No. 4.3: FT-IR spectra of simvastatin/HP-β-CD complex by physical mixture.

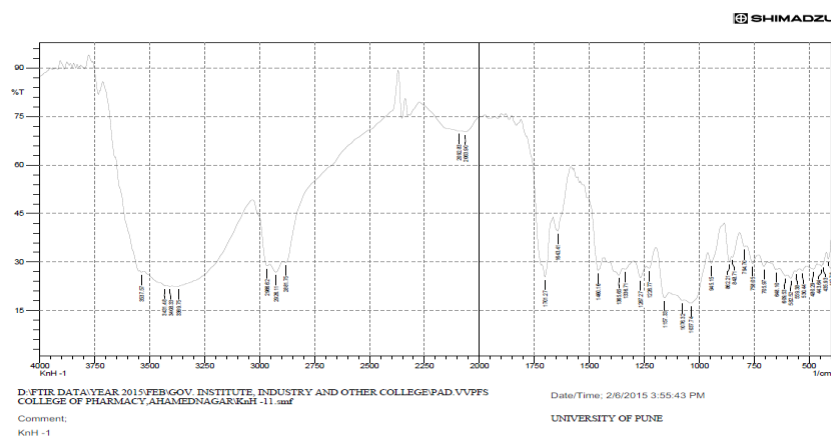


Fig. No. 4.4: FT-IR spectra of simvastatin/HP-β-CD complex by kneading method.

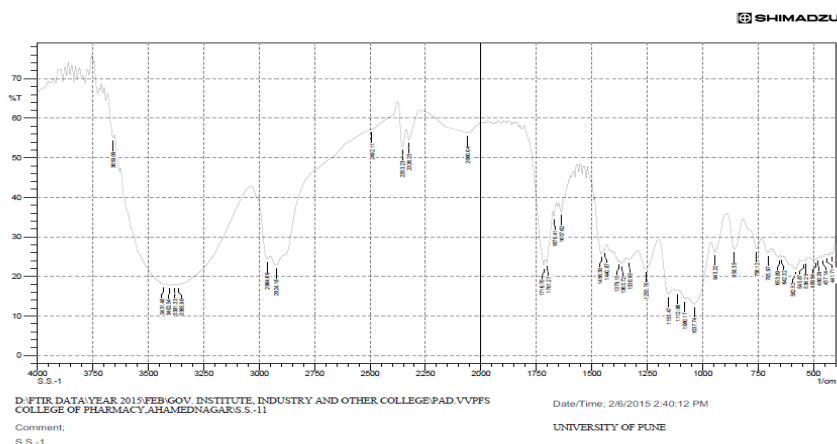


Fig. No. 4.5: FT-IR spectra of simvastatin/HP- β -CD complex by spray drying method.

The spectrum of simvastatin shows absorption bands in 3549 cm^{-1} (for O-H stretching vibrations), 3007 , 2958 and 2874 cm^{-1} (for C-H stretching vibrations), 1701 cm^{-1} (stretching vibrations for ester and lactone carbonyl functional group) (fig. no. 4.1). The FT-IR spectra of HP- β -CD shows absorption bands at 3410 (for O-H stretching vibrations), 3381 , 3358 , 3327 cm^{-1} , 2926 cm^{-1} (for C-H stretching vibrations), 1643 cm^{-1} (for C=O stretching vibrations), 1153 cm^{-1} , 1080 cm^{-1} (for C-H, C-O stretching vibrations) (fig. no. 4.2). The FT-IR spectra of simvastatin/HP- β -CD inclusion complex by the physical mixture (fig. no. 4.3) and kneading method (fig. no. 4.4) does not show significant difference from the spectra of the simvastatin and HP- β -CD. For the inclusion complex by the spray drying the FT-IR spectrum was similar to that of HP- β -CD and characteristic peaks of simvastatin almost completely disappeared. This may be due to inclusion complexation of drug into the HP- β -CD cavity. The FT-IR results corresponding to results of DSC and PXRD, indicating inclusion complex formation between simvastatin/HP- β -CD. Results shows that C=O group of lactone ring of simvastatin might be involved in simvastatin/HP- β -CD inclusion complex formation.

3.5 FILED EMISSION SCANNING ELECTRON MICROSCOPY (FESEM)

The FESEM is qualitative method used to study the structural aspects of raw material i.e. cyclodextrin, drugs and the products obtained by different methods of preparation such as physical mixing, solution complexation, kneading method, co-evaporation and others.^[25] The Filled emission scanning electron microscopy of simvastatin, HP- β -CD, simvastatin/HP- β -CD inclusion complex by physical method, kneading method and spray drying represented in fig. 5.1 to 5.5.

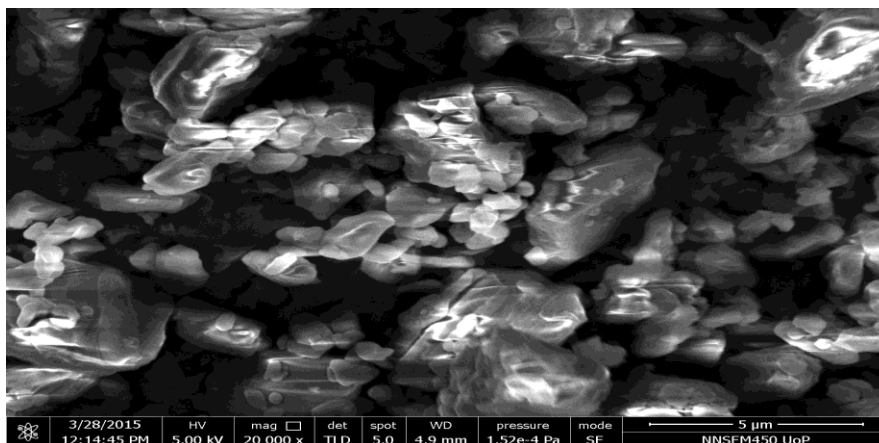


Fig. No. 5.1: Filed emission scanning electron microscopy (FESEM) of simvastatin.

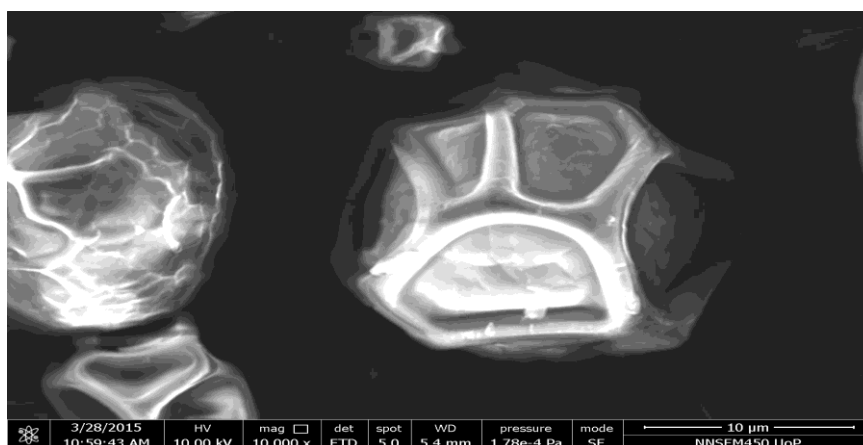


Fig. No. 5.2: Filed emission scanning electron microscopy (FESEM) of HP-β-CD.

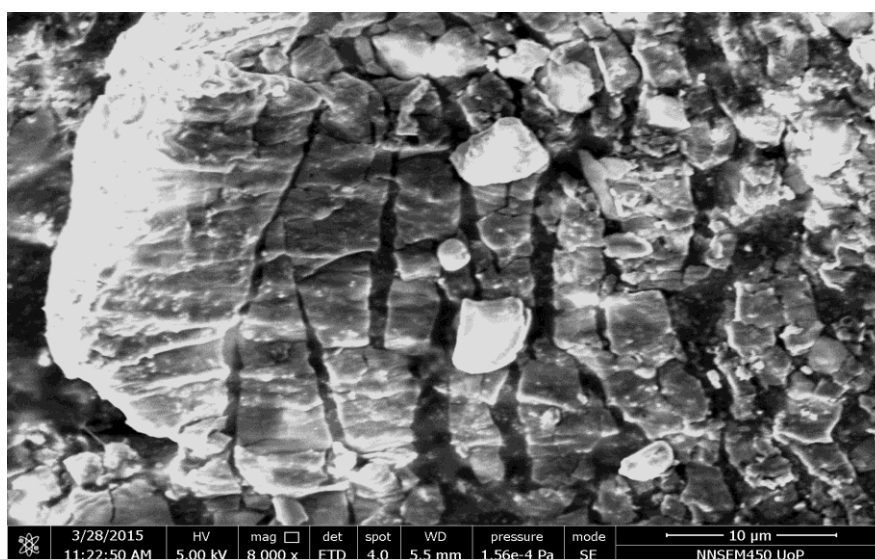


Fig. No. 5.3: Filed emission scanning electron microscopy (FESEM) of simvastatin/HP-β-CD by physical mixture.

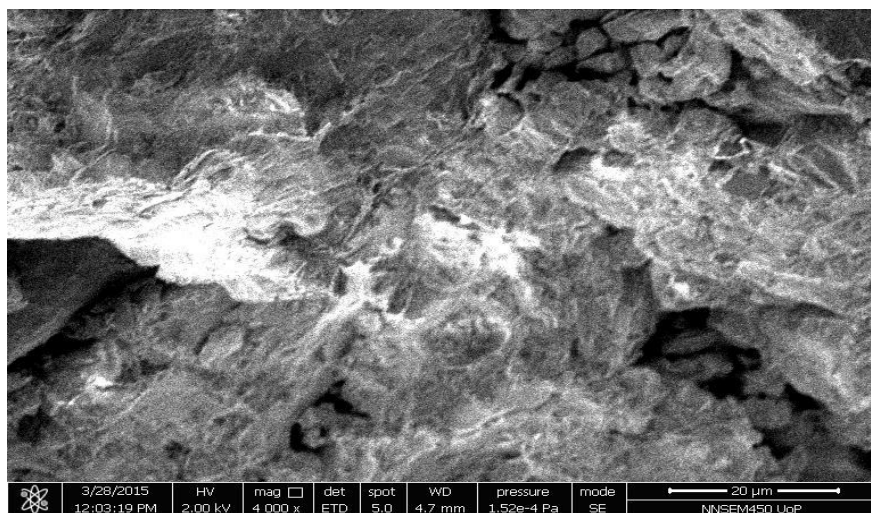


Fig. No. 5.4: Filed emission scanning electron microscopy (FESEM) of simvastatin/HP-β-CD by kneading method.

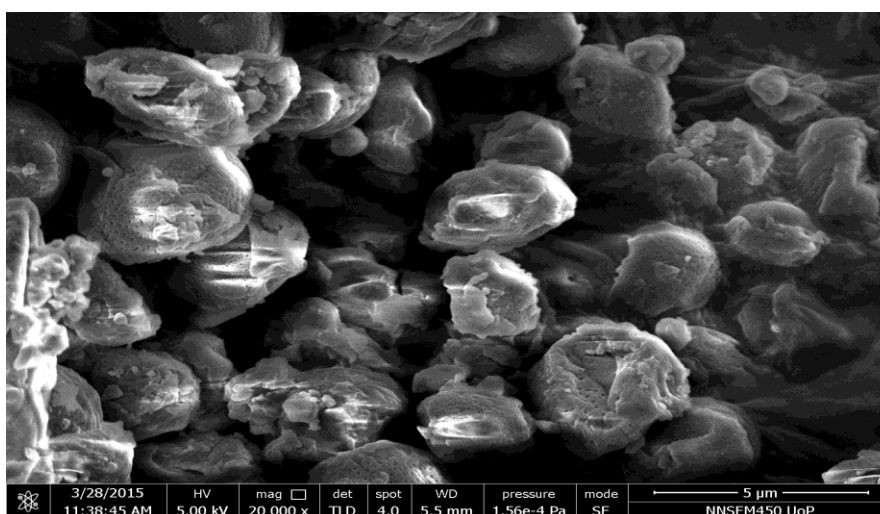


Fig. No. 5.5: Filed emission scanning electron microscopy (FESEM) of simvastatin/HP-β-CD by spray drying.

The FESEM photographs of simvastatin consist of the typical large crystals in many different sizes (fig. no. 5.1). The FESEM of HP-β-CD consisted of hollow spherical particles (fig. no. 5.2). The simvastatin/HP-β-CD complex by the physical mixture (fig. no. 5.3) and kneading method (fig. no. 5.4) revealed irregular shaped crystals, showed both crystalline components. However, the simvastatin/HP-β-CD inclusion complex appeared as a oval and uniform shape like structure crystals and was quite different from the sizes and shapes of HP-β-CD and simvastatin (fig. no. 5.5), which confirms the formation of the simvastatin/HP-β-CD inclusion complex, revealing apparent interaction in the solid state.

3.6 DRUG CONTENT AND AQUEOUS SOLUBILITY

The drug content within all inclusion complexation methods found to be $95 \pm 2\%$ ($n = 3$). Aqueous solubility of Simvastatin at the end of 48 h in phosphate buffer solution (pH 6.8) indicated an aqueous solubility of $27.6 \pm 1.5 \mu\text{g/ml}$ (Table 1). The spray dried simvastatin exhibited approximately a 3-fold increase ($87.1 \mu\text{g/ml}$) in drug solubility, compared with simvastatin. The spray dried simvastatin with β -CD increase in drug solubility to $218.4 \mu\text{g/ml}$. Simvastatin with HP- β -CD spray dried significantly increase in drug solubility to $279.2 \mu\text{g/ml}$.

Table No. 1: Aqueous solubility of SV in phosphate buffer solution (pH 6.8) at $37 \pm 0.5^\circ\text{C}$ after 48 h Solubility data at the end of 48 h expressed as mean \pm SD for $n = 3$.

Formulation	Aqueous solubility ($\mu\text{g/ml}$)
Simvastatin	27.6 ± 1.24
Spray dried simvastatin	87.1 ± 5.96
Spray dried simvastatin with β -CD	218.4 ± 4.64
Spray dried simvastatin with HP- β -CD	279.2 ± 7.59

3.7 DISSOLUTION STUDY

The dissolution characteristics of simvastatin and inclusion complex with HP- β -CD by the physical mixture, kneading method and spray drying given in fig. no. 6, shows the dissolution profiles of different formulations in 60 min.

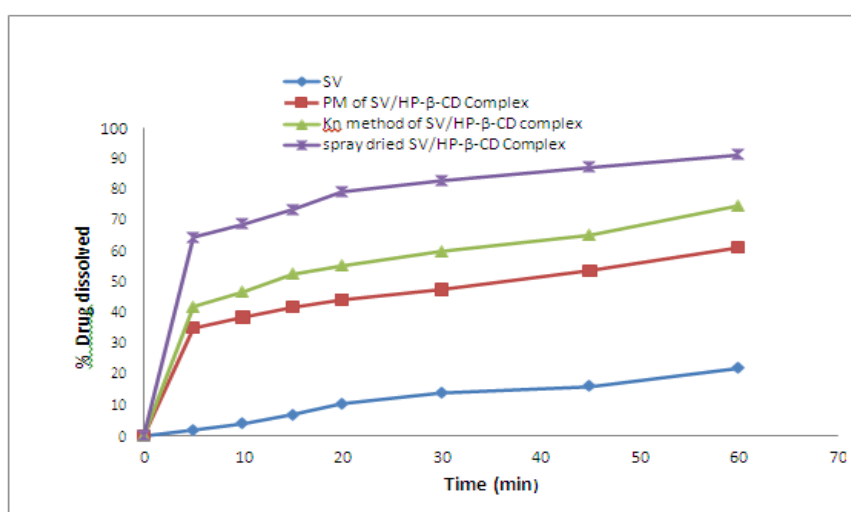


Fig. No. 6: Dissolution profiles of SV, physical mixture of SV/HP- β -CD, kneading method of SV/HP- β -CD and spray dried of SV/HP- β -CD complex in phosphate buffer, PH 6.8.

From fig. no. 6 the dissolution of spray dried simvastatin/HP- β -CD dramatically improved, as compared with simvastatin. The rate of dissolution improved as the simvastatin, simvastatin inclusion complex with HP- β -CD by physical mixture, kneading method and spray drying. Based on dissolution profiles from these.

Studies the higher dissolution rate of spray dried simvastatin/HP- β -CD complex was may be mainly attributed to the simvastatin complexation with HP- β -CD during spray dried process. The inclusion complex formation between simvastatin/HP- β -CD probably leads to the intermolecular hydrogen bond formation between simvastatin and HP- β -CD by spray drying process.

4. CONCLUSION

The results of the studies indicated the formation of the simvastatin/HP- β -CD inclusion complex at 1:1 molar ratio formation with stability constant 105 M^{-1} . In present study, the physical mixture, kneading method and spray drying applied to formation of inclusion complex between simvastatin and HP- β -CD. The phase solubility diagram classified as the type A at 25°C , indicating the inclusion complex formation at 1:1 stoichiometric ratio. In the DSC study no endothermic peak observed in the inclusion complex of spray drying. The PXRD does not consist characteristic diffraction pattern corresponding to the simvastatin in inclusion complex. The FT-IR results shows the presence of intermolecular hydrogen bonds between the simvastatin and HP- β -CD, it indicating formation of the amorphous form. FESEM consist formation oval and uniform shape crystals in inclusion complex compared with the simvastatin, indicated modification in morphology. The aqueous solubility and dissolution study indicates that the dissolution rate tremendously increased in the spray dried simvastatin/HP- β -CD complex compared to physical mixture, kneading method and drug. It was found that the spray drying could be useful method for the preparation of inclusion complex of simvastatin with HP- β -CD and it shows that the solubility and dissolution rate tremendously increased by complexation between simvastatin and HP- β -CD.

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