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Research Article

DESIGN, CHARECTERIZATION AND EVALUATION OF INCLUSION COMPLEXES OF POORLY SOLUBLE ATORVASTATIN CALCIUM

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ABSTRACT

In this present research work, an effort was made to enhance the solubility of Atorvastatin calcium which is insoluble in water and leads to low bioavailability due to incomplete absorption, the primary approach is to increase the solubility and dissolution of Atorvastatin calcium by complexation techniques using β-cyclodextrins and HPβ-CD. Complexes were prepared using physical mixture, kneading and Spray drying method. Inclusion complexes Atorvastatin calcium withβ-CD and HP-βCD formedin1:1molar ratios. All prepared complexes showed improved dissolution when compared to drug alone and this was characterized by XRD, DSC and SEM studies. The markedly improvement in dissolution rates of Atorvastatin calcium was observed with AF_7 and AF_{13}prepared by kneading method and spray drying method with β-CD and $AF_{10}$and AF_{16}prepared by kneading method and spray drying method with HP-βCD respectively. The dissolution rates increases as concentration of β-CD and HP-βCD increases. The rate of dissolution is higher in complexes prepared with spray drying method compared with physical mixture and kneading method. Hence it can be conclude that the inclusion complexes prepared by spray drying method was showed enhanced solubility improved dissolution rate.

Keywords: Atorvastatin calcium, Solubility enhancement, Cyclodextrins, Inclusion complex.

INTRODUCTION

Atorvastatin, as a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme. A (HMGCoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, an early rate- limiting step in cholesterol biosynthesis andit is currently used as calcium salt for the treatment of hypercholesterolemia[1].Orally administered drugs completely absorb and exhibit good bioavailability only when they are soluble in gastric medium. Poorly water soluble drugs show poor intestinal absorption due to limited solubility leading to inadequate bioavailability. Thus, improvement in the solubility profile of poorly water soluble drug is desirable in order to increase their absorption and resultant bioavailability[2,3]. Several methods have been proposed and used to improve the bioavailability of such drugs including micronization, salt formation[4,5]. Use of metastable polymorphs; solvent disposition; selective adsorption on insoluble carriers; solid dispersion[6,7] complexation with cyclodextrins and solute solvent complexation[8]. Cyclodextrin (CDs) are potential carriers for achieving such CDs are cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity that can accommodate a variety of lipophilic drugs. However, the natural CDs, in particular β-cyclodextrin (βCD), have limited solubility in water and their complexes with lipophilic water-insoluble drugs often result in precipitation of drug from complexes [9]. Hence, chemical modifications often made to enhance and expand the functionalities of CDs. This includes the βCD derivatives like 2- hydroxypropyl- βCD (HPβCD), randomly methylated βCD, sulfobutylether- βCD and maltosyl- βCD. The complexation efficiency and solubilizing effect of CDs in aqueous solution also increase by addition of water soluble polymers [0-12]. Present investigation was undertaken to enhance the solubility, dissolution rate and bioavailability of poorly soluble Atorvastatin Calcium through formation of an inclusion complex with βCD, HPβCD.

Phase solubility studies for Atorvastatin Calcium:

The most widely used approach to study the phase solubility method described by Higuchi and Connors.50 mg of Atorvastatin calcium was added to aqueous solution containing 0-10mM β-cyclodextrin and transferred to 25ml stoppered conical flask. The mixture was shaken for 72 hrs. Aliquots of 2 ml were withdrawn and filtered immediately using 0.45µ nylon disc filter. The filtered samples were diluted with pH 6.8 Buffer suitably and assayed for Atorvastatin calcium by measuring absorbance at 246 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 equation.

The slope is obtained from the initial straight line portion of the plot of Atorvastatin calcium against cyclodextrins concentration, and S_o is the equilibrium solubility of Atorvastatin calcium in pH 6.8 Buffer[13].

MATERIALS AND METHODS

Methods used in preparation of inclusion complexes of Atorvastatin Calcium:

Physical mixture:

The Atorvastatin Calcium with β-CD and HPβ-CD in different molar ratios (1:1M, 1:2M and 2:1) were mixed in a mortar for about one hour with constant trituration, passed through sieve # 100.Then stored in a desiccators over fused calcium chloride[14,15].

Kneading method:

The Atorvastatin Calcium with βCD and HP-βCD in different molar ratios (1:1M, 1:2M and 2:1) were taken. Cyclodextrin placed in the mortar, small amount of 50 % methanol is added while triturating to get slurry like consistency. Then slowly drug was incorporated into the slurry and trituration is further continued for 1 hour. The mass was then air dried at 25°C for 24 hrs, pulverized and passed through sieve # 100. The prepared complexes were stored in desiccators over fused calcium chloride[14,15].

Spray drying method:

The inclusion complex of Atorvastatin Calcium with βCD and HP-βCD was prepared by spray drying method. Thedrug with β-CD were dissolved in isopropyl alcohol (IPA) and distilled water separately. Both the solutions were mixed with magnetic stirrer for 30min.The resulting solution was fed to mini spray dryer (Labultima-222,Mumbai) and sprayed in the chamber from the 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 drug with HPβ-CD.The product thus obtained was collected, packed and doubly wrapped in an aluminum foil and stored in a desiccators till further use[14,15].

Evaluations of inclusion complexes of Atorvastatin Calcium:

Drug Content Estimation of Atorvastatin Calcium:

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 analyzing for the Atorvastatin Calcium content spectrophotometrically at 246 nm [16].

Invitro dissolution study of Atorvastatin Calcium:

Dissolution of Atorvastatin Calcium pure drug and inclusion complexes (100 mg) was assessed at 37 ^{0}C±0.5 ^{0}C. Using USPII (USP XXII) dissolution test apparatus (Paddle),in 900 ml of pH 6.8bufferas the dissolution medium and at a rotation speed of 75 rpm. Aliquots, each of 5 ml, from the dissolution medium were withdrawn at time intervals of 5, 10, 15,…,upto 30 min and replenished by an equal volume of fresh dissolution medium to maintain sink condition. The samples withdrawn were filtered (0.45 µm) and analyzed for drug release by measuring its absorbance at 246 nm using pH 6.8bufferas blank. The process was repeatedfor3 times[17].

Characterization of Atorvastatin Calcium inclusion complexes:

X-ray Diffraction Study:

The Atorvastatin Calcium ofX-ray diffraction study was done to study the powder characteristics of and its inclusion complexes with β-cyclodextrin and HPβ-cyclodextrin. The x-ray diffractograms were obtained by Philips diffractometer (PW 1140) and Cu-Kα radiation diffractograms were runs at a scanning speed of 2^o/min and a chart speed of 2^o/ 2cm/ 2θ[13].

Differential Scanning Calorimetry (DSC):

The DSC study of Atorvastatin Calcium was done using a Perkin Elmer Pyris (Shelton, CT) and mettler equipped with an intercooler 2P cooling accessory. The samples of 4mg were placed in standard aluminum pans and sealed with a lid. Heating scans by 10^{o}C/min were applied with a nitrogen purge of 20ml/min, over a temperature range of 30^{o}C to 285^{o}C. An empty aluminum pan was used as reference for the analysis [13].

Scanning electron microscopy (SEM):

The surface morphology of Atorvastatin Calcium was determined using scanning electron microscope (SEM) (HITACHI. S-3000N-Japan) operated at an accelerating voltage 20 kV (lament current of 1.75 l beam current of 30 – 40 Ma, probe current of 250 pA). Samples were prepared by mounting 0.5 mg of powder onto a5 mm silicon wafer fixed with graphite tape toan aluminum stub. The powder was then sputter-coatedfor 40 s at beam current of 38 – 42 mA with a 200 A ° layer of palladium alloy[13].

RESULTS AND DISCUSSIONS

Phase solubility study of Atorvastatin Calcium:

The phase-solubility diagrams were of AL according to Higuchi and Connors. The effect β-CD and HP-βCD on the solubility of Atorvastatin calcium in pH 6.8 phosphate buffer solution was investigated at 25^{o}C. It was found that the solubility of Atorvastatin calcium was increased markedly by complexation with β-CD and HP-βCD. The phase solubility analysis of Atorvastatin calcium indicated the formation of a 1:1 molar inclusion complexes with complexing agents at pH 6.8 buffer solution at a given cyclodextrin concentration range (0 to 10 mM). The apparent solubility constant (Kc) obtained from the slope of the linear phase solubility diagrams was found to be 248.015M^{-1} for Atorvastatin Calcium -β-CD complexes and 312.68M^{-1} for Atorvastatin calcium with HP-βCD complexes. This value of stability constant (Kc) indicated that complexes formed is quite stable shown in fig. 1 and 2.

Evaluation of Atorvastatin calcium inclusion complexes:

Drug Content Uniformity of Atorvastatin calcium:

The percentage of drug content uniformity of Atorvastatin calcium was found to be between 95.75% to 104.62% in formulations between AF_1 to AF_{18}.

Dissolution study of Atorvastatin calcium:

The drug dissolution data of Atorvastatin calcium with β-CD and HPβ-CD of AF_1 to AF_{18} are given in table 2 and 3. It is evident that the complexes prepared by physical, kneading and

spray drying method exhibited a faster dissolution when compared to pure drug alone. The percent of drug release from all inclusion complexes (AF_1 to AF_{18}) was found in the range of 82.53 to 99.56% within the 30 minutes, where as the pure drug of Atorvastatin Calcium exhibited only 48.52%.The inclusion complexes of Atorvastatin Calcium with β-CD, exhibited drug release in pH 6.8 buffer in the following order, 86.45%, 98.56%, 82.23%, 88.65%, 99.56%, 84.12%, 90.23%, 99.23% and 86.42% from formulations AF_1, AF_2, AF_3, AF_7, AF_8,AF_9,AF_{13}, AF_{14}, andAF_{15}, respectively in 30 minutes. The inclusion complexes of Atorvastatin Calcium with HP-βCD, exhibited drug release in pH 6.8 buffer in the following order, 89.56%, 99.12%, 84.21%, 92.45%, 98.54%, 87.28%, 95.56%, 99.52% and 89.35%. from formulations AF_4, AF_5, AF_6, AF_{10}, AF_{11}, AF_{12}, AF_{16}, AF_{17}&AF18respectively in 30 minutes. The dissolution rates increases as the concentration of β-CD and HP-βCD increases. The rate of dissolution is higher in complexes prepared with spray drying method compared with physical mixture and kneading method shown in Fig. 3 to 7.

X-ray Diffraction Study of Atorvastatin calcium:

The diffractograms of Atorvastatin calcium and β-CD exhibited a series of intense peaks, which is an indicative of their crystalline nature and HP-βCD exhibited a series of less intense peaks, which is an indicative of their less crystalline nature compared with the β-CD. X-RD pattern of physical mixture, formulation $AF_1$and AF_4 it is simply the superimposition of each component of the drug and complexing agent indicating there is no formation of new structure. The inclusion complex prepared by the kneading method, formulation $AF_7$and AF_{10}showed a diffraction pattern quite similar to that of the physical mixture method. The diffractograms obtained from Spray drying method, formulation $AF_{13}$and AF_{16} showed less peaks with low intensity. This indicating that, the complex prepared by the Spray drying method which is less crystalline than the complexes prepared by physical mixture and kneading method shown in Fig. 8 and 9.

DSC study of Atorvastatin calcium:

The DSC thermograms of Atorvastatin calcium, β-CD and HPβ-CD and corresponding there cyclodextrin inclusion complexes are shown in Fig. 10 and 11. The DSC thermo gram of Atorvastatin Calcium showed an endothermic peak at 167.8°C corresponding to its melting point. The thermogram of β-CD showed a very broad endothermic effect, which attains a maximum around 80 to 90°C due to the release of water molecules and peak at 90.14°C corresponding to its melting point. The thermogram of HPβ-CD showed an endothermic effect, which attains a maximum around 100 to 130°C due to the release of water molecules and peak at 129.17°C corresponding to its melting point. The thermograms of Atorvastatin Calcium with β-CD (1:1 M) prepared by physical mixture method and kneading method i.e., formulation AF_1 and AF_7, respectively showed endothermic peaks at 60-148.1°C. This may be due to shift of characteristic peak of Atorvastatin calcium which was observed at 167.8°C, indicates a strong interaction between the drug and complexing agentβ-CD. In case of Atorvastatin Calcium with β-CD complexes (1:1 M) prepared the spray drying method formulation AF_{13}, exhibited a very broad endothermic peak at 68.8°C instead of 167.8°C indicating strong interaction between drug and complexing agent at 1:1 molar ratio. The thermograms of Atorvastatin Calcium with HPβ-CD (1:1 M) prepared by physical and kneading method i.e., formulationAF_4 and AF_{10}, respectively showed endothermic peaks at 140-171.1°C. This may be due to shift of characteristic peak of Atorvastatin Calcium which was observed at 167.8°C, indicates a strong interaction of drug and HPβ-CD. In case of Atorvastatin Calcium with HPβ-CD complexes (1:1 M) prepared by spray drying method formulation AF_{16}, exhibited a very broad endothermic peak at 97.5 and 119.2°C indicating strong interaction between drug and HPβ-CD.

SEM Study of Atorvastatin calcium:

The SEM is used to assess the microscopic aspects of the drug Atorvastatin calcium and complexes like β-CD and HP-βCD. The Atorvastatin calcium was characterized by the presence of crystalline particle of a regular size. The Crystals of drug mixed with crystals of complexing agents were seen that adhering there surface of each other. The SEM Photographs of β-CD and HPβ-CD is slightly crystalline in nature. The SEM photographs formulation AF_{16} of spray dried inclusion complexes showed the characteristic morphology of the preparations, generally obtained in this method that are small sized particles tending to aggregation with each other, indicating the existence of an amorphous nature product with presence of single component in the complexation thus suggesting the maximum complexation shown in Fig. 12.

CONCLUSION

Inclusion complexes of Atorvastatin calcium with βCD and HP-βCD formedin1:1 complexesby three methods such as physical mixture, kneading and spray drying method. All prepared complexes showed improved dissolution when compared to drug alone and this was characterized by XRD, DSC and SEM studies. The dissolution of Atorvastatin calcium from inclusion complexes prepared with HPβ-CD (1:1M) by physical mixture, kneading method spray drying method was found to be higher than the pure drug and spray drying method showed higher rate of dissolution compared with the physical mixture, kneading method. Hence from the above results it can be conclude that the spray drying method was best method given perfect inclusion complex with β-CD and HPβ-CD.

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Table 1: Formulation details of inclusion complex of Atorvastatin Calcium.

Methods	Drug to carrier For AC	Ratio For AC	Code For AC
Pure Drug	AC	-	AF_0
Physical Mixture	APM:βCD	1:1	AF_1
	APM:βCD	1:2	AF_2
	APM:βCD	2:1	AF_3
	APM:HP-βCD	1:1	AF_4
	APM:HP-βCD	1:2	AF_5
	APM:HP-βCD	2:1	AF_6
Kneading	AKM:βCD	1:1	AF_7
	AKM:βCD	1:2	AF_8
	AKM:βCD	2:1	AF_9
	AKM:HP-βCD	1:1	AF_{10}
	AKM:HP-βCD	1:2	AF_{11}
	AKM:HP-βCD	2:1	AF_{12}
Spray Drying	ASD: βCD	1:1	AF_{13}
	ASD: βCD	1:2	AF_{14}
	ASD: βCD	2:1	AF_{15}
	ASD: HP-βCD	1:1	AF_{16}
	ASD: HP-βCD	1:2	AF_{17}
	ASD: HP-βCD	2:1	AF_{18}

Table 2: Consolidated table for *in vitro* drug releaseof (AF_0 to AF_9) AC and its formulations in pH 6.8 Buffer.

Time in min	In vitro dissolution study of AC and its formulations in pH 6.8 Buffer.									
	AF_0	AF_1	AF_2	AF_3	AF_4	AF_5	AF_6	AF_7	AF_8	AF_9
5	8.64	14.56	20.56	12.62	16.64	23.21	14.42	16.52	21.54	13.52
10	17.54	28.55	42.83	24.85	31.64	45.12	26.12	30.25	44.52	25.85
15	24.82	44.75	63.45	38.45	46.45	66.32	41.52	45.12	65.24	39.98
20	33.28	57.64	82.54	53.62	59.68	84.62	55.56	58.24	84.23	54.23
25	42.78	72.65	96.23	68.96	74.86	98.65	70.86	74.23	97.56	70.52
30	48.52	86.45	98.56	82.23	89.56	99.12	84.21	88.65	99.56	84.12

Table 3: Consolidated table for *in vitro* drug releaseof (AF_{10} to AF_{18}) AC and its formulations in pH 6.8 Buffer.

Time in min	In vitro dissolution study of AC and its formulations in pH 6.8 Buffer.								
	AF_{10}	AF_{11}	AF_{12}	AF_{13}	AF_{14}	AF_{15}	AF_{16}	AF_{17}	AF_{18}
5	18.23	25.34	16.58	18.56	23.24	15.24	20.42	26.89	17.54
10	33.54	48.56	27.54	32.51	46.52	27.34	35.54	49.52	28.56
15	48.62	68.54	43.62	48.94	67.52	42.72	51.52	69.42	45.52
20	62.82	87.54	56.94	62.14	86.12	56.23	63.85	89.24	58.23
25	76.52	98.56	72.53	76.46	98.21	73.86	78.95	99.52	73.46
30	92.45	98.54	87.28	90.23	99.23	86.42	95.56	99.52	89.35

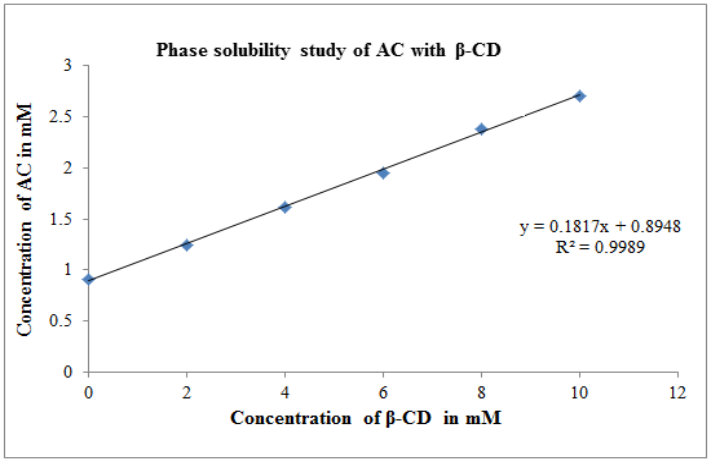

Figure 1: The phase solubility graph of Atorvastatin Calcium with β-CD complex

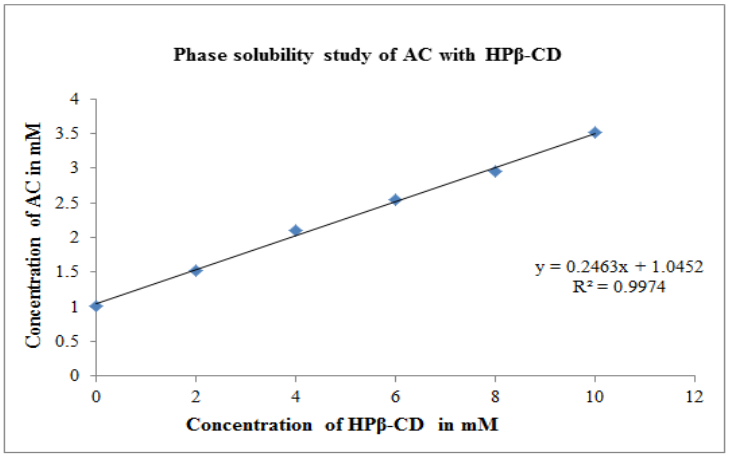

Figure 2: The phase solubility graph of Atorvastatin Calcium with HPβ-CD complex

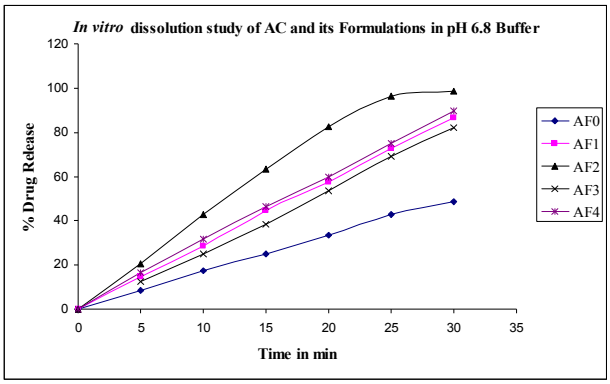

Figure 3: *In vitro* dissolution study of Pure drug AF_0 andAF_1, AF_2, AF_3, AF_4 Formulations

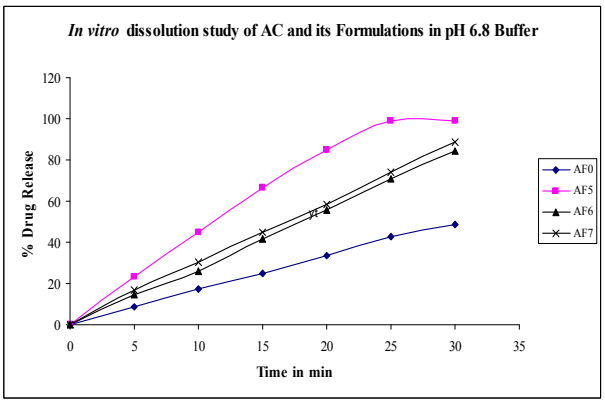

Figure 4: *In vitro* dissolution study of Pure drug AF_0 andAF_5, AF_6, AF_7, Formulations

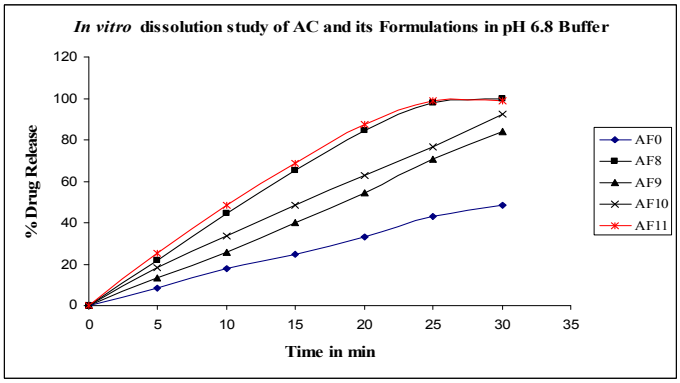

Figure 5: *In vitro* dissolution study of Pure drug AF_0 andAF_8 AF_9, AF_{10}, AF_{11} Formulations

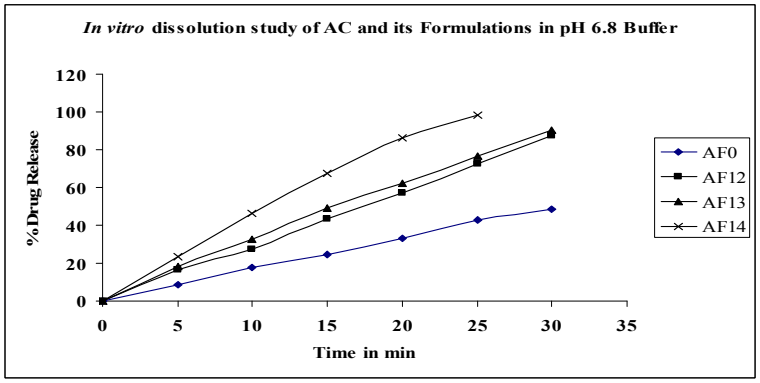

Figure 6: *In vitro* dissolution study of Pure drug AF_0 andAF_{12} AF_{13}, AF_{14}, Formulations

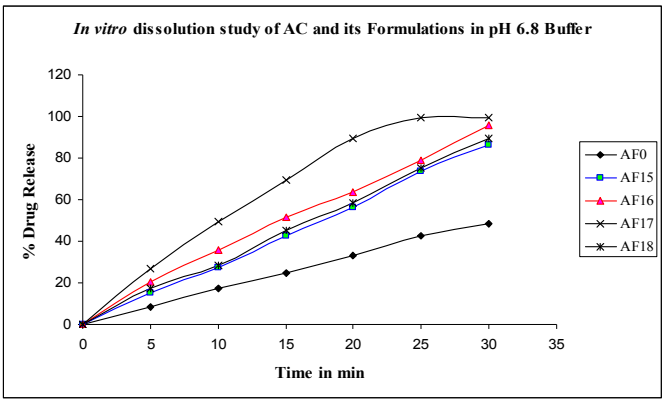

Figure 7: *In vitro* dissolution study of Pure drug AF_0 andAF_{15} AF_{16}, AF_{17}, AF_{18}, Formulations

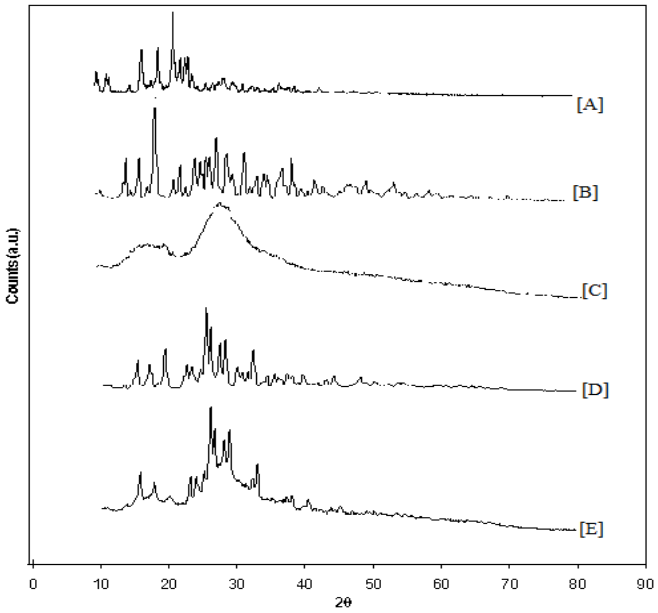

Figure 8: **XRD Thermogram of (A) Atorvastatin pure (B) Pure β-CD (C) HPβ- CD (D) Physical mixture AC- β-CD (E) Physical mixture AC- HPβ-CD.**

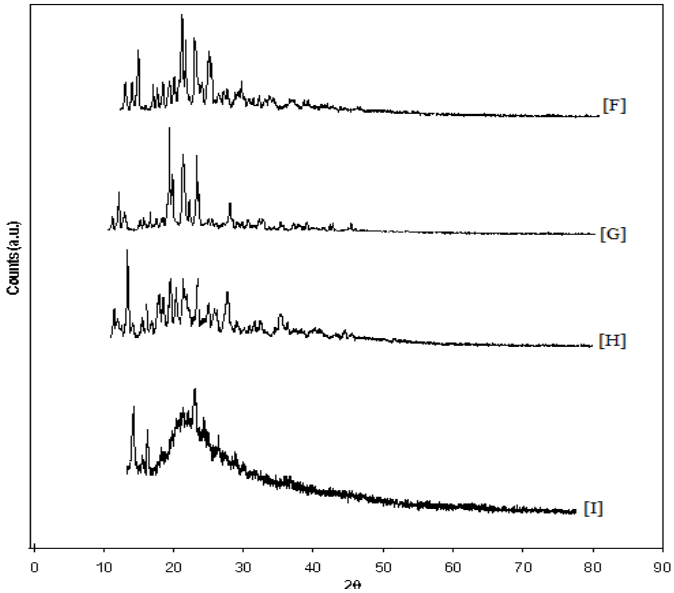

Figure 9: **XRD Thermogram of (F) Kneading method AC- βCD (G) Kneading AC-Hβ-CD (H) Spray drying method AC- β-CD (I) Spray drying method AC- HPβ-CD.**

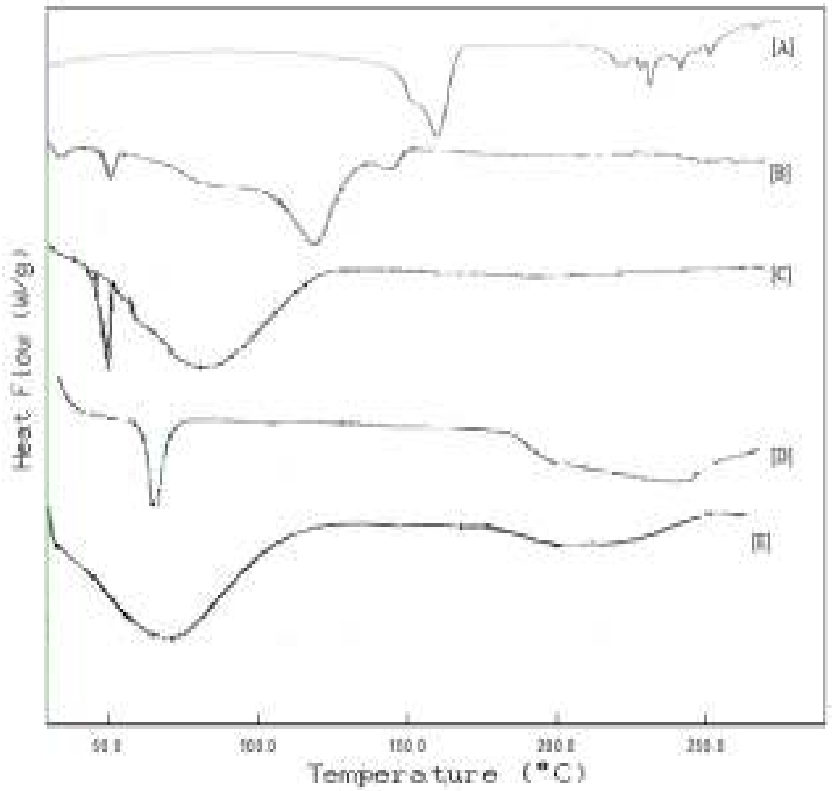

Figure 10:DSC Thermogram of (A) Atorvastatin pure (B) Pure β-CD (C) HPβ- CD (D) Physical mixture RAC- β-CD (E) Physical mixture AC- HPβ-CD.

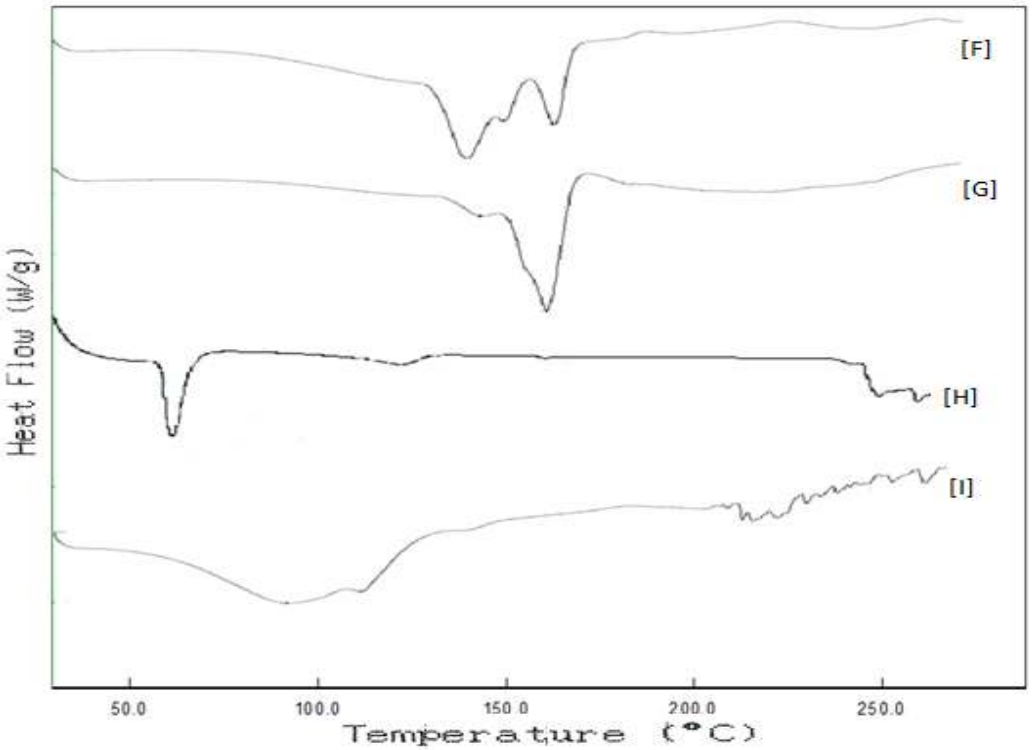

Figure 11:DSC Thermogram of (F) Kneading method AC- βCD (G) Kneading AC-Hβ-CD (H) Spray drying method AC- β-CD (I) Spray drying method AC- HPβ-CD.

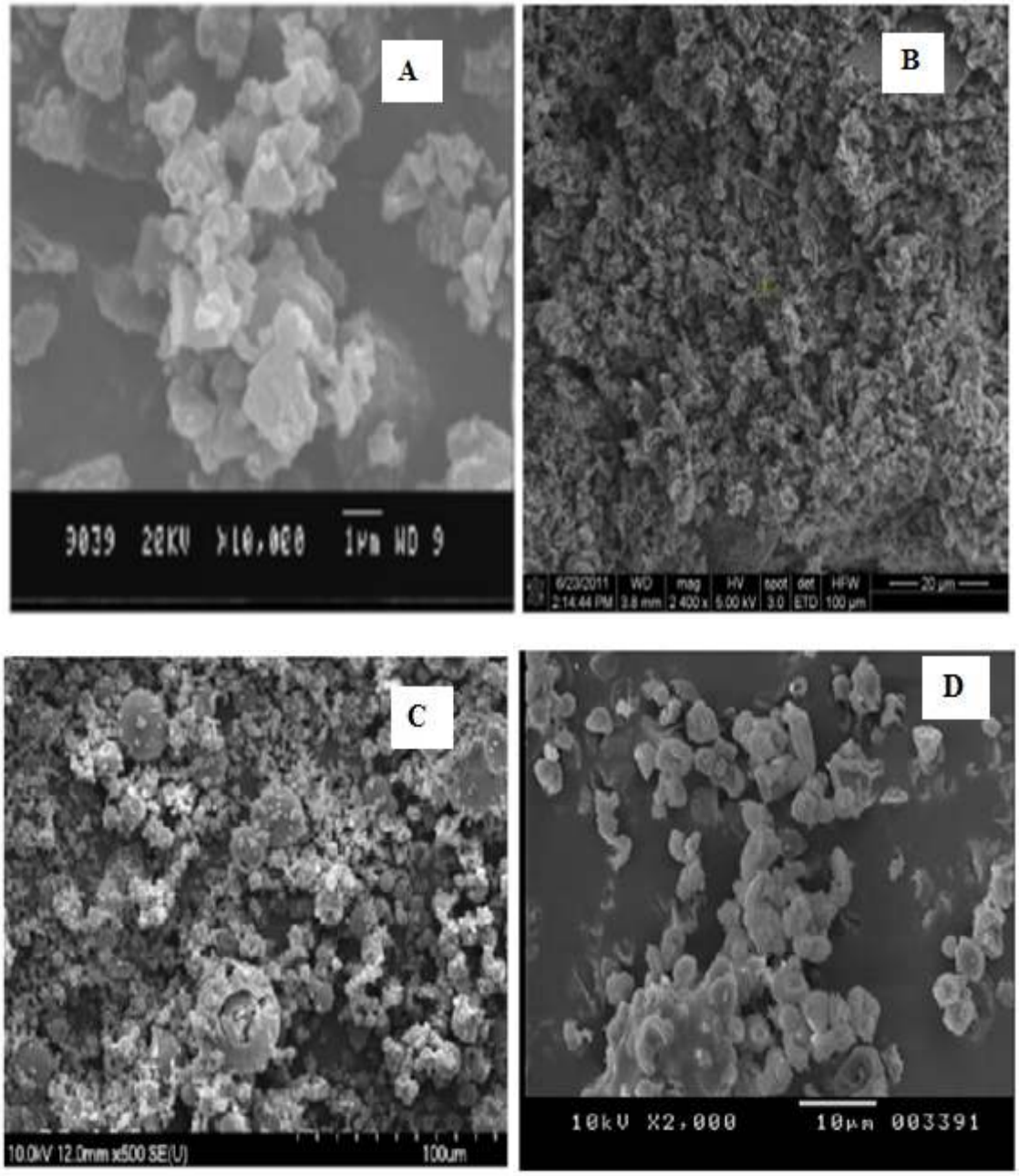

Figure 12: Scanning Electron Microscopy Images of (A) Atorvastatin pure (B) Pure β-CD. (C) HPBCD (D) Formulation AF16.

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