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SOLID DISPERSIONS, INCLUSION COMPLEXES, AND FAST-RELEASE TABLETS OF ATORVASTATIN: SOLID STATE CHARACTERIZATION, DISSOLUTION BEHAVIOR, AND *IN VIVO* PHARMACOKINETIC EVALUATION

Sanjay Dey†, Bhaskar Mazumder

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India.

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Corresponding Author:

Sanjay Dey †

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India.

E-mail: sanju1980dey@gmail.com

ABSTRACT

The objective of the present study was to prepare solid dispersion (SD) and inclusion complexes of atorvastatin (ATV) using different hydrophilic carriers (polyethylene glycol (PEG 4000 and PEG 6000), polyvinyl pyrrolidone (PVP K30), and D-mannitol) and β-cyclodextrin (β-CD), respectively to improve the aqueous solubility and dissolution rate. Different drug: carrier ratios (1:1, 1:2, and 1:3) were used and their effects on the dissolution performance were studied. The physical state and drug-carrier interaction in solid state were analyzed by infrared spectroscopy, differential scanning calorimetry, and X-ray diffraction. The dissolution rate of ATV from tablets containing β-CD inclusion complex was compared with conventional tablets without β-CD. *In vivo* pharmacokinetic studies were performed on rabbits to compare the *in vivo* performance between tablets with or without inclusion complex. Improvement in the drug dissolution rate was observed in SDs and inclusion complexes as compared to the physical mixtures. Inclusion complexes of ATV with β-CD gave a better drug release profile as compared to among other formulations. B-CD–based tablets exhibited a significant higher ATV dissolution than did conventional tablets prepared without β-CD. A shorter t_{max} and greater C_{max} and AUC were obtained by using tablets prepared from inclusion complex as compared to conventional tablets. It can be concluded that the oral bioavailability of ATV could be improved by administration of tablets containing β-CD inclusion complex as compared to conventional tablets prepared without β-CD.

Keywords: Atorvastatin; solid dispersion; inclusion complex; phase solubility study, phase solubility study

INTRODUCTION

Most of the newly discovered drug entities, in spite of high therapeutic activity, have poor aqueous solubility, leading to poor absorption in the gastrointestinal tract (GIT), results in poor bioavailability [1]. An important prerequisite for the absorption of a drug from GIT is that it must be present in aqueous solution. The poor dissolution characteristics of relatively insoluble drugs have still remains a problem to the pharmaceutical industry because the dissolution rate could be the rate-limiting process in the absorption of a drug from a solid dosage form. Therefore, it is a great challenge to the formulator to develop efficient, economical, reliable, and scalable method to increase the oral bioavailability of poorly water-soluble drugs. Different approaches have been used to tackle this situation like: particle size reduction through microsizing and nanosizing, salt formation, solubilization, etc. All these approaches suffer from certain drawback. Last few decades, formulation of solid dispersion using hydrophilic inert carrier and formation of inclusion complex of drug with β-cyclodextrin (β-CD) have been most widely used to enhance the dissolution rate and bioavailability of poorly water-soluble drugs [2-7]. Solid dispersion (SD) refers to the dispersion of one or more active ingredients in a hydrophilic inert carrier matrix at molecular level.

Increase in the dissolution of drug leading to improved bioavailability through the incorporation of drug into the inert solid carrier has been reported [2].
When the solid dispersion is exposed to the aqueous medium, the carrier gets dissolved first; later, the drug is release in the form of fine colloidal particles, results in increased effective surface area, which leads to enhance dissolution rate and bioavailability of poorly-water soluble drugs.

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The use of several inert carriers for the formulation of SD has been reported. Poly ethylene glycol (PEG), polyvinyl pyrrolidone (PVP), and D-mannitol are the most widely used carriers. PEG has been used extensively as water soluble carrier because of high solubility in water, low melting point, wettability, rapid solidification rate, low toxicity, and low cost [2, 8-11]. However, at higher drug concentrations, the drug is often present in the crystalline form within the PEG dispersion or it recrystallizes over time, resulting in unstable formulations with low dissolution rates [12-13]. The ability to stabilize the amorphous sate of drugs due to inhibition of drug recrystallization as well as a rapid solidification rate and low toxicity favour the carriers PVP for the preparation of solid dispersion [14-16]. PVP is used as solubilizing, complexing, and dispersing agent in pharmaceutical preparation [17]. It is well tolerated physiologically, readily soluble in water, and has been used for increasing the dissolution and oral absorption of many water insoluble drugs [18-21]. D-mannitol, a water soluble polyol, has been reported to use as vehicle in the preparation of SD of many poorly soluble drugs [22-25]. It has attracted much attention because of its high melting point (166-168^{o}C), low toxicity, high aqueous solubility, low hygroscopicity, and physiological acceptance.

β-CD, a cyclic oligosaccharide, has been widely used in pharmaceutical industry for increasing solubility/dissolution and, hence the bioavailability of poorly water-soluble drugs due to economic reasons (low cost) [26-27]. β-CD owing to their configurations, have a cylinder-shaped, electron-rich, internal hydrophobic cavity and a hydrophilic external surface. The lipophilic cavity enables β-CD to form noncovalent inclusion complexes with wide variety of poorly water soluble compounds in aqueous solutions by spatial entraphysical mixtureent of some or whole part of molecule, whereas the hydrophobic outer surface render these inclusion complexes water soluble [28].

Atorvastatin (ATV) is a member of lipid lowering agent. It is a potent inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme (HMG-CoA) reductase which catalyzes the conversion of HMG-CoA to mevalonate, an early rate-determining step in cholesterol biosynthesis [29]. ATV is currently used as calcium salt for the treatment of hypercholesterolemia. It is insoluble in aqueous solution of pH 4 and below. It is very slightly soluble in water and pH 7.4 phosphate buffer. It is comes under Biopharmaceutical Classification System II (BCS II) category. It is rapidly absorbed at the relevant intestinal pH after oral administration [30-31]. Its absolute bioavailability has been reported 12%, results in low plasma concentration is achieved following oral administration of 40 mg dose [32].

The objective of the present study was to evaluate the enhancing effect of different hydrophilic carriers and β-CD on dissolution behavior of ATV. The formation of such complex was confirmed by the phase solubility study, infrared (IR) spectroscopy, differential scanning colorimetry (DSC), and x-ray diffraction (XRD) studies. The aim of this work was to study the potential of inclusion complexes for development of fast-releases tablets of ATV using β-CD. The dissolution profile of ATV using β-CD tablets was compared with that of a reference ATV tablet without β-CD.

2.0. MATERIALS AND METHODS

2.1. Materials

Atrovastatin calcium (ATV) was obtained form Cipla Ltd, Mumbai, India. PEG 4000 and PEG 6000 were purchased form Merck, Mumbai, India. D-mannitol and polyvinyl pyrrolidone K30 (PVPK-30) were purchased from Qualigens fine Chemicals, Mumbai, India. β-CD was procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Sodium starch glycolate was procured from Mepro Pharma, Vadodara, India. Double-distilled water was obtained through a MilliQ system (Waters, USA). All other chemicals were of reagent grade and used without further purification.

2.2. Phase Solubility Studies

Phase solubility studies were carried out to investigate the effect of different carriers on the solubility of ATV, using method reported by Higuchi and Connors [33]. Double-distilled water containing no carrier and aqueous solution of different carriers of different concentration (5%, 10%, and 15% w/v) were added to excess amounts of ATV and shaken at 30^{o}C for 24 hrs. After equilibrium, the solution were filtered using Whatmann$^{(R)}$ No. 1 filter papers and diluted suitably to determine the concentration of ATV by UV-Visible spectrophotometer (UV-1700, Shimadzu, Tokyo, Japan) at 242 nm. The graph of concentration of ATV was plotted against the concentration of different carriers. The apparent stability constant for the complex was determined from the graph using following equation:

$$K_s = {}^{\text{Slope}}/_{S_o\,(1 - \text{Slope})} \qquad (1)$$

Where slope obtained from the graph and S_o is the equilibrium solubility of ATV in water.

2.3. Preparation of Physical mixture

Physical mixtures of drug with different carriers (PEG 4000, PEG 6000, D-mannitol, and PVPK-30) were prepared separately at three different ratios (1:1, 1:2, and 1:3). Accurately weighed 50 mg drug was taken and mixed thoroughly with 50, 100, and 150 mg of individual carriers using a spatula. Finally, all the mixtures were passed individually through a sieve no. 60 having aperture size of 250 µm. The prepared mixtures were stored in a desiccator until further use.

2.4. Preparation of SD

SD of ATV was prepared by solvent evaporation method using different ratio (1:1, 1:2, and 1:3) of carriers (PEG 4000, PEG 6000, D-mannitol, and PVPK-30) individually. The drug was weighed accurately (50 mg) and dissolved in 70% v/v ethanol to obtain a clear solution. Individual carriers was added to the solution and dispersed well. The solvent was evaporated at room temperature with occasional stirring. The resultant residue was dried under vacuums for 3 h and stored overnight in a desiccator. Finally, the mass obtained was crushed, pulverized, and sifted through sieve no. 60 having mesh size of 250 µm. The prepared solid dispersion was stored in desiccators until further use.

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2.5. Preparation of Inclusion Complex

Inclusion complex of ATV with β-CD was prepared using the similar method as solid dispersion was prepared. Inclusion complexes of ATV were prepared separately at three different ratios 1:1, 1:2, and 1:3.

2.6. Dissolution Studies

Dissolution studies of pure drug, physical mixtures, SDs, and inclusion complex were conducted on a USP Type 2 apparatus. The dissolution medium was 900 ml of 0.1M hydrochloric acid (pH 1.2) maintained at 37±0.5°C and stirred at 75 rpm by means of adjustable constant speed motor. Powder containing equivalent to 10 mg of ATV was introduced in the dissolution medium, and the time was recorded (time 0). At predetermined time intervals 5 mL of samples were withdrawn and the same volume of fresh dissolution medium, maintained at 37±0.5°C was added to the flask to maintain constant volume. The samples were analysed using UV-Visible spectrophotometer (UV-1700, Shimadzu, Tokyo, Japan) at 241 nm. Dissolution studies of each formulation were carried out in triplicate.

2.7. Characterization of Physical mixture, Solid Dispersion, and Inclusion Complex

2.7.1. Infrared Spectroscopy

Infrared (IR) spectra of the pure ATV, physical mixtures, SDs, and inclusion complex were recorded with a System 2000 instrument (Perkin-Elmer, Corp., Norwalk, CT, USA) by the KBr disc method from 4000 to 400 cm^{-1}.

2.7.2. Differential Scanning Calorimetry

The DSC thermograms of different samples were investigated on Diamond differential scanning calorimeter (Perkin Elmer, USA) using 2-3 mg samples. Samples were sealed in aluminum pans and scanned from 30 to 400°C at a heating rate of 15°C/min in an atmosphere of nitrogen gas.

2.7.3. X-Ray Diffractometry

X-ray diffractometry of different powder samples were investigated on Rigaku Denki diffractometer (MiniFlex 2027, Tokyo, Japan) with copper target and nickel filter at 30 kV, 5 mA current, 4°/min scanning speed, and 5°-40° (2θ) range.

2.8. Preparation of Fast-Release Tablets

On the basis of dissolution characteristics of solid dispersions and inclusion complexes, the fast-release tablets were prepared using inclusion complex of ATV and β-CD in the ratio of 1:3. ATV:β-CD (40 mg in the ratio of 1:3 drug:polymer), sodium starch glycolate (5.0 mg), and dicalcium phosphate (68.52 mg) were manually blended in a mortar for 10 min.

Then the powder blend was mixed with 5.4 mg of talc and 1.08 mg of magnesium stearate using laboratory glass mortar and pastel and mixed uniformly for 10 min. Conventional tablets of atorvastatin without β-CD were also prepared in a similar manner using the same excipients. Tablets were compressed with a compression force of 12kN using a single punch-tableting machine (Kilburns, Allahabad, India), equipped with 6-mm concave punches.

2.9. Tablet Assay, Physical Evaluation, and Dissolution Study

The tablets were assayed for drug content using methanol as extracting solvent, and the samples were analyzed spectrophotometrically (Shimadzu 1700, Kyoto, Japan) at 241 nm. Tablets were also evaluated for hardness (n = 6), friability (n = 6), weight variation (n = 10), disintegration time (n = 3), and thickness (n = 10). Dissolution studies of the tablets were performed using the same method as mentioned in Section 2.6.

2.8. Pharmacokinetic Study

Six New Zealand white stains rabbits of either sex weighing 1.5-2.2 kg were used for *in vivo* bioavailability study. They were kept on a standard diet and fasted for 12 h prior to oral administration of the drug with free access of water. Tablets containing β-CD or without β-CD was administered was administered orally through feeding tube to each group of three rabbits.

The blood samples were collected from the marginal ear vein into the previously heparinised tubes. The blood samples were withdrawn at 0 (blank plasma) and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 12 h after administration of the drug. The blood samples were immediately centrifuged at 7500 × g for 10 min. The plasma thus obtained was stored at -20°C until analysis.

2.9. HPLC Condition

The HPLC system equipped with quaternary pump (Water™ 600), 7725i rheodyne manual injector and UV-Visible detector of module 2998 and empower-II software. Chromatographic separation was achieved using Hypersil BDS C-18 analytical column (250 × 4.6 mm I.D.), which was packed with 5 µm particles with a mobile phase consisting of phosphate buffer (pH 4.0)-acetonitrile-methanol (40:40:20 v/v). The mobile phase was filtered, degassed and pumped at a flow rate of 1 mL min^{-1}.

2.10. Determination of ATV in Plasma

The liquid-liquid extraction technique was used as an extraction technique on sample preparation. Blood samples were collected in disposable glass tubes (100 × 16 mm) and centrifuged at 4500 × g for 5 min. The serum samples were stored at -80°C until analysis. Hundred microliter of plasma sample was taken in a 2 mL glass centrifuge tube; 10 µL of internal standard (Diltiazem; 50 µg mL^{-1}) was added and the mixture was vortex for 10 seconds using multi-pulse vortexer (Glas-COL, USA). The samples were subjected to liquid-liquid extraction using 1.5 mL of acetonitrile as extracting solvent. After vortex mixing for 10 min and centrifugation (5 min at 10000 rpm at 4°C), the organic phase was removed and evaporated to dryness under stream of nitrogen at 25 psi at 40°C for 10 min. The residue was reconstituted in 100 µL of methanol and the sample was vortex for 1 min using multi pulse vortexer at speed of 100 without pulser. A volume of 20 µL was injected into the HPLC system.

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2.12. Determination of Pharmacokinetic Parameter and Statistical Assessment

The area under plasma concentration time curve ($AUC_{0-\alpha}$), the maximum plasma concentration (C_{max}), and the time to reach the maximum plasma concentration (T_{max}) were selected as parameters for pharmacokinetic evaluation. The C_{max} and T_{max} were determined directly by visual inspection of the experimental data of plasma concentration vs. time [34]. The AUC_{0-12hr} was calculated by the linear trapezoidal rule and the $AUC_{0-\infty}$ was estimated by the following equation: $AUC_{0-\infty} = AUC_{0-t} + C_{last}/K_{el}$. The K_{el} was estimated by the performing log linear regression on the concentration versus time data points. The $t_{1/2}$ was calculated by using the equation of $0.693/K_{el}$. Mean residence time (MRT) was calculated as area under the first moment curve (AUMC) divided by $AUC_{0-\infty}$. AUMC was determined using a plot of plasma concentration multiplied by time (Ct) versus time and calculation of its area under the curve. Variation in pharmacokinetic parameters was tested statistically by using one-way analysis of variance. In all tests, a probability value $p<0.05$ was considered statistical significant.

3.0. RESULTS AND DISCUSSION

Different hydrophilic carrier like polyethylene glycol (PEG 4000 and PEG 6000), PVP K30, and D-mannitol were chosen for the present studies, as these are highly water soluble, non-toxic, and have the capacity to enhance dissolution rates of insoluble drugs. β-CD was selected in present study as these forms inclusion complex with highly lipophilic drugs and render them hydrophilic, results in enhance dissolution rates.

3.1. Phase Solubility Studies

Table 1 shows the effects of different carriers on the solubility of ATV in water at 30°C. The saturation solubility of the drug was enhanced in all cases, as compared to the control, pure drug. It was also observed that increasing carrier weight fraction results in an increase in drug solubility. The solubilising effect of β-CD in the ratio of 1:3 (ATV:carrier) was higher than those of the other systems.

Table 1: Effects of different carriers on the solubility of ATV in water at 30°C.

	Solubility of ATV (mg/mL)		
	1:1	1:2	1:3
PEG 4000	1.56	1.83	2.17
PEG 6000	0.78	0.94	1.11
PVP K30	0.50	0.56	0.61
D-mannitol	1.11	1.67	1.83
β-cyclodextrin	1.89	2.22	2.67
ATV		0.28	

Fig. 1 shows the phase solubility diagrams of ATV with different carriers. In all systems, the linear stability curve could be classified as type A_L type, indicating the system were first order in nature [33]. The linear relationship also implied that dilution of solution of ATV-carrier complex during administration into the body would not cause precipitation of ATV regardless of the extent of dilution. Table 2 shows the apparent stability constants (K_s) of the ATV-carrier complexes. The stability constant value can be referred to as the strength or magnitude of complexation of ATV to the different carriers [35]. Hence, β-CD and D-mannitol have the strongest complex forming ability with ATV, while that of the PVP K30 can be considered negligible. As for PEG 4000 and PEG 6000 the values wee intermediate between those PVP K30 and β-CD/D-mannitol.

Table 2: Apparent solubility constant (K_s) and types of solubility curve ATV-carriers complexes determined by solubility method in water at 30°C.

Carriers	K_s (M^{-1})	Type
PEG 4000	20.85	A_L
PEG 6000	14.06	A_L
PVP K30	6.42	A_L
D-mannitol	23.75	A_L
β-cyclodextrin	23.52	A_L

3.2. Dissolution Studies

Fig. 2 represents the release of ATV from physical mixtures and SDs prepared by using PEG 4000 and PEG 6000. The release profile portrait that the release of ATV from PEG 6000 SD is more as compared to PEG 4000 SD at drug:carrier ratio of 1:3. Fig. 3 shows the release profile of PVP K30 and D-mannitol SDs and their physical mixtures. The results of the dissolution study of pure ATV showed that only 14% of ATV was released in 2 hr. The possible reasons for this poor drug dissolution are low aqueous solubility and poor wettability of ATV. During the first minutes, the dissolution process was characterized by a higher concentration of carriers around the drug particles. This affected the diffusion layer that surrounded the drug particles and improved the micro-environmental solubility, resulting in fast drug dissolution. Release profile shows there are few differences in dissolution behaviour between pure ATV and the physical mixtures.

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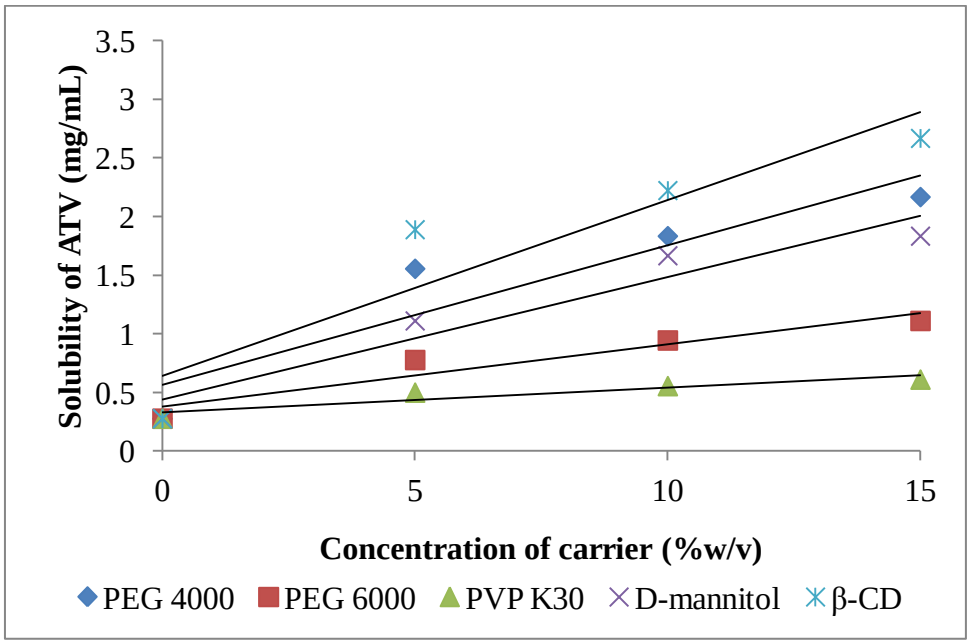

Figure 1: Phase solubility diagrams of ATV-carrier system in water at 30°C.

The dissolution rate of all processed powders is higher than that of ATV alone, and this increase depends on the amount of carrier and drug present in the powder. As the amount of carrier increased, the dissolution rate of ATV also increased. Perhaps, the slight enhancement of dissolution is achieved in the physical mixtures that can be attributed to two concomitant effects, the improved wettability of the physical mixture due to the presence of hydrophilic component and possible formation of hydrodynamic layer surrounding the ATV particle in the acid medium. Both effects lead to the improvement of ATV dissolution rate of ATV.

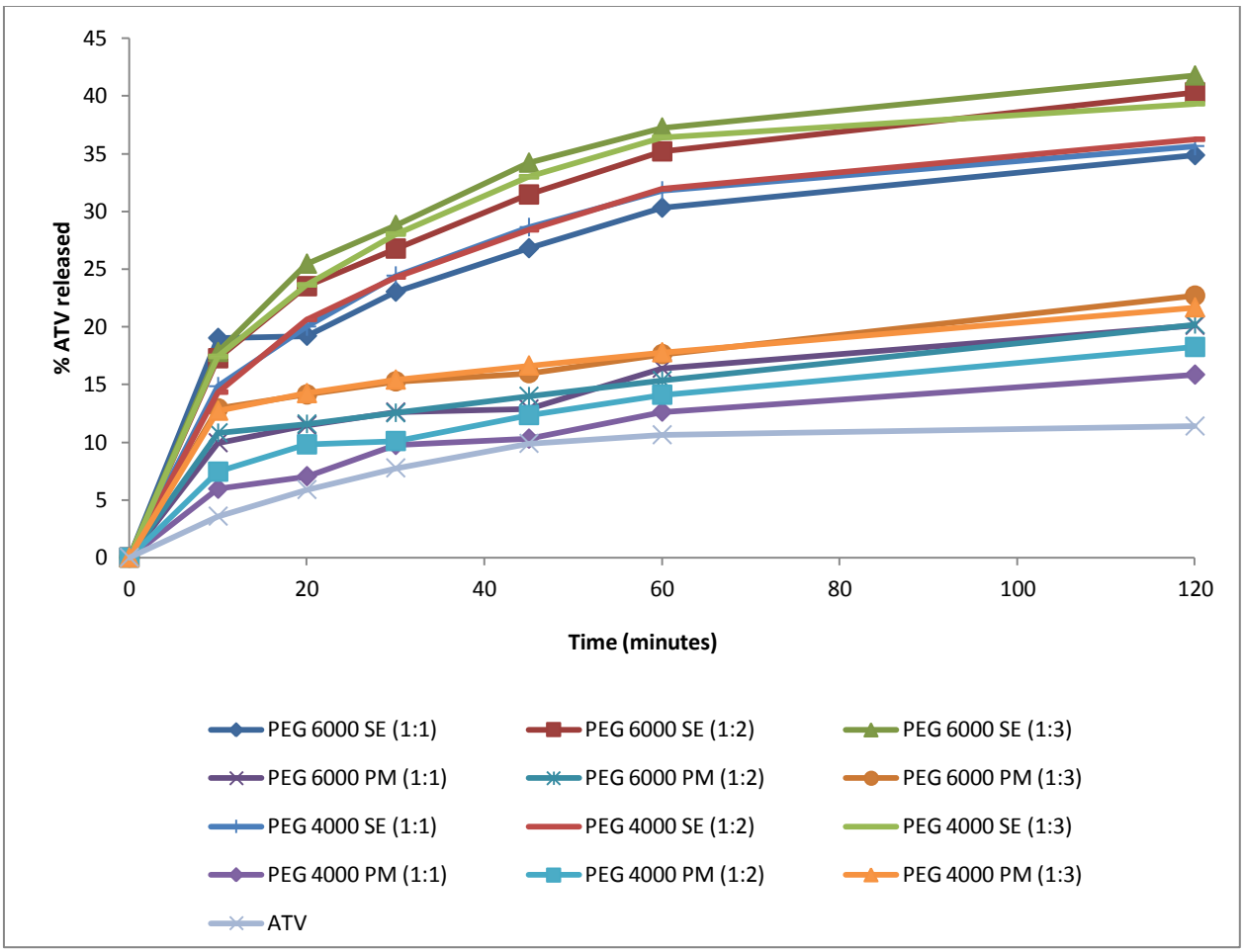

Figure 2: ATV solid dispersions: effects of PEG 4000 and PEG 6000 content and method of preparation on the dissolution behaviour. The mean of three determinations were presented.

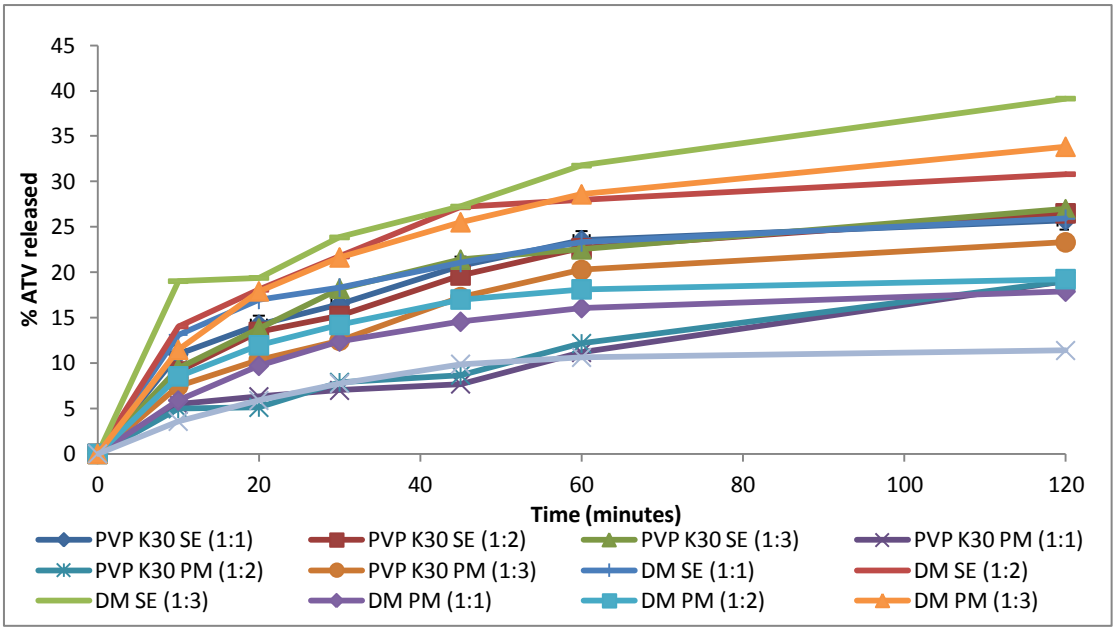

Figure 3: ATV solid dispersions: effects of PVP K30 and D-mannitol content and method of preparation on the dissolution behaviour. The mean of three determinations were presented

The ATV SDs showed better dissolution performance over corresponding physical mixture and the pure drug. This may be due to an improved wettability of the drug particles, a significant reduction in particle size during the formation of SD. Three different drug: carrier weight ratios (1:1, 1:2, and 1:3) were used to access the effect of increasing carrier concentration on the release of ATV from SD and physical mixture. In all the cases, an increase in weight fraction of the carrier resulted in an improvement in the rate and extent of drug dissolution. The possible reasons for this include enhance of ATV dissolution by dissolved amounts of the carrier [36] & a decrease in the particle size of the drug in the carrier, with an increase in carrier concentration.

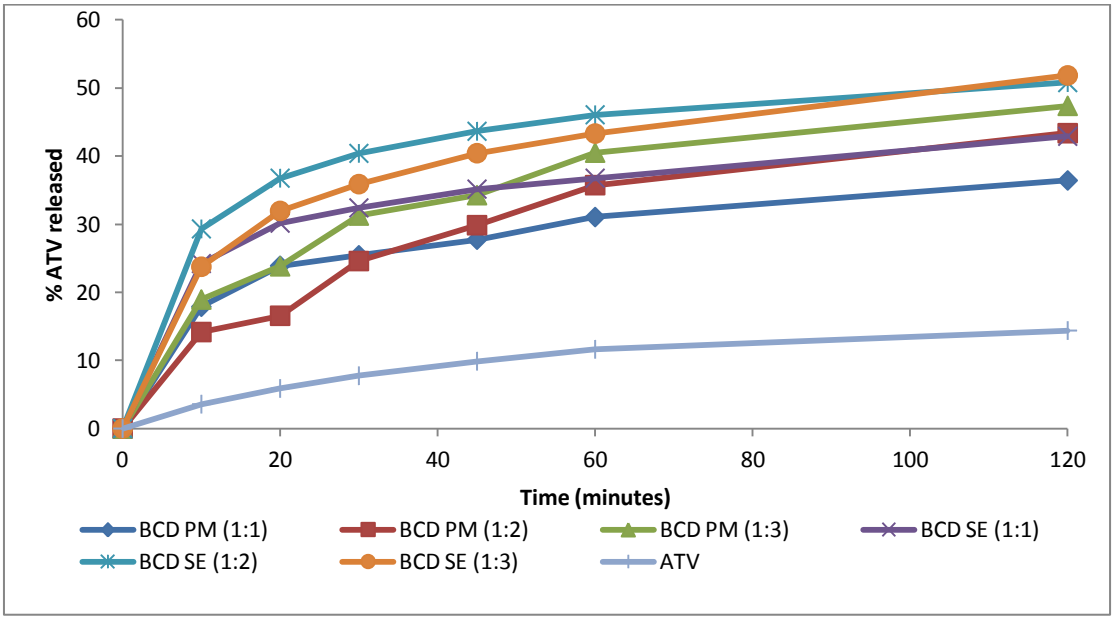

Figure 4: ATV inclusion complexes: effects of β-CD content and method of preparation on the dissolution behaviour. The mean of three determinations were presented.

Fig. 4 depicted that the ATV release from inclusion complex is better as compared to the physical mixture. The higher rate and extent of dissolution of inclusions complex may be attributed to the hydrophilic effects of β-CD, which reduced both the hydrophobicity of ATV as well as the interfacial tension between ATV and the dissolution medium. Moreover, the amount of ATV dissolved from the SD was approximately 3.6 times higher compared to the pure drug. This phenomenon may be due to inherent differences between the carriers in terms of intrinsic rates of dissolution and hydration, and possible complex formation between drug and carrier or decrease in crystallinity of the coprecipitated drug.

In addition, complexation is capable of reducing the particle size and crystallinity of ATV, thus contributing further to the increased rate of dissolution. From the release profile it can be observed that all SDs and complex achieved both a higher rate and extent of release as compared with their respective physical mixtures. Within the first few minutes, ATV release from different SDs increased quickly, and then the release decreased after reaching the saturation solubilities, but not with inclusion complexes. This phenomenon could be ascribed to the recrystallization of ATV from the dissolution medium, this suggesting that the inclusion complex is more capable of stabilizing the supersaturated solution, possibly due to the better fit of the ATV molecule in the β-CD cavity. Every value of the presented curves is the mean of three experiments. Standard deviation bars are omitted to avoid overlapping as but, for all values, the standard error was less than 2%.

3.3. Infrared Spectroscopy Studies

The interaction between the drug and the carrier often leads to identifiable changes in the infrared profile of SDs [37]. The infrared spectra of physical mixture, SDs, and inclusion complex were compared with the standard spectrum of ATV. The infrared spectra of ATV, carriers, β-CD, the physical mixtures, the SDs, and the inclusion complex in the same molar ratio are illustrated in Fig. 5. The presence and absence of characteristic peaks associated with specific structural characteristic of the drug molecules were noted. The infrared spectra of ATV showed sharp bands at 3365cm^{-1} (OH stretching), 3056 cm^{-1} (C-HO stretching alcoholic group), 1650 cm^{-1} (C=C bending), 1557 cm^{-1} (C=O stretching amide group), 1315 cm^{-1} (C-N stretching), 1220 cm^{-1} (C-F stretching), 1109 cm^{-1} (C-O stretching), and 1015 cm^{-1} (O-H bending).

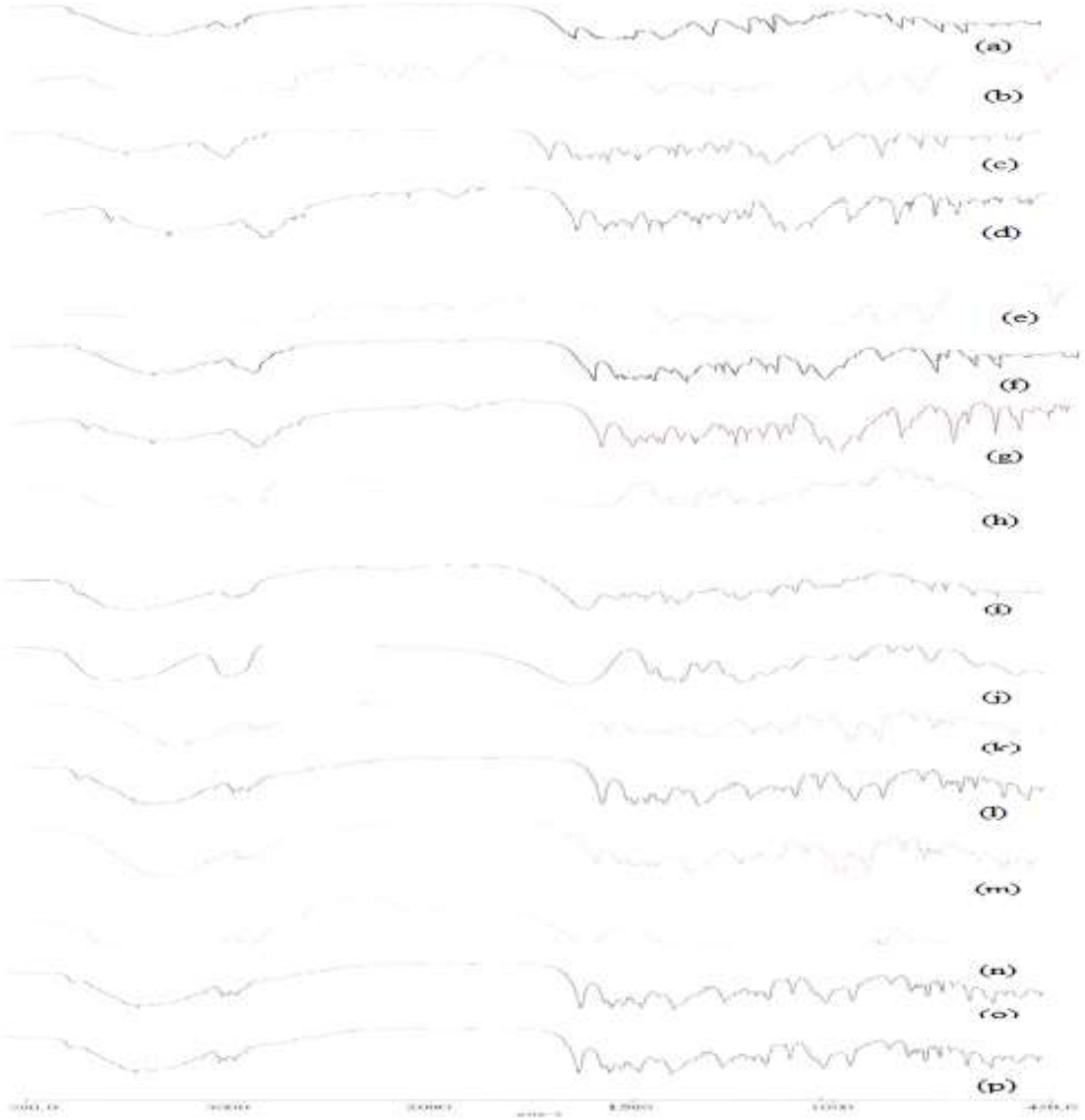

Figure 5: FTIR spectra of a) pure ATV; b) PEG 4000; c) ATV/PEG 4000 physical mixture; d) ATV/PEG 4000 solid dispersion; e) PEG 6000; f) ATV/PEG 6000 physical mixture; g) ATV/PEG 6000 solid dispersion; h) PVP K30; i) ATV/PVP K30 physical mixture; j) ATV/PVP K30 solid dispersion; k) D-mannitol; l) ATV/D-mannitol physical mixture; m) ATV/D-mannitol solid dispersion; n) β-CD; o) ATV/β-CD physical mixture; p) ATV/β-CD inclusion complex.

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Due to the similarity of the molecular structure, PEG 4000 and PEG 6000 showed similar absorption spectra, in which characteristic broad peak of O-H stretching vibration from 3300 to 3600 cm^{-1}, C-H stretching of OC_2H_5 groups from 2800 to 2900 cm^{-1}, and C-O stretching from 1000 to 1200 cm^{-1} were observed. The spectra of SDs and physical mixtures were equivalent to the addition spectrum of PEG 4000 or PEG 6000 and ATV. These results indicate absence o well-defined interaction between ATV and PEG 4000 or PEG 6000. Although it could be expected to have hydrogen bonding between the hydrogen atom of the OH of the ATV and one of the ion pairs of the oxygen atom in PEG 4000/PEG 6000.

The IR spectra of PVP K30 is characterized by the absorption of C=O stretching band at 1650 cm^{-1} and C-N band at 1287 cm^{-1}. The IR spectra of physical mixture showed bands similar to that of both drug and PVP K30. SDs prepared by solvent evaporation method at 1:1 ratio displayed similar spectra as that of physical mixture at the corresponding ratio indicating absence of interaction between ATV and PVP K30.

The IR spectra of pure D-mannitol showed sharp peaks between 3400 cm^{-1} and 3200 cm^{-1} that are characteristic of the OH stretching vibrations. Another set of sharp peaks were obtained between 3000 and 2800 cm^{-1}, characteristic of the C-H stretching vibrations. In the physical mixture, the spectrum is the superposition of those of the pure products with attenuation of the ATV peaks, showing no significant differences from the respective spectra of the pure components. However, the IR spectrum of the SDs prepared by using D-mannitol exhibited some significant differences. For the SDs, the ATV peak mostly disappeared, probably due to hydrogen bonding, occurred between ATV and D-mannitol in SDs.

The spectrum of pure β-CD showed the vibration of free –OHs between 3000 and 3400 cm^{-1} and those bound –OHs at 2760 cm^{-1}. In the physical mixture and inclusion complex, the ATV peak mostly disappeared and the excess of free β-CD was still visible, suggesting that some interaction probably hydrogen bonding, occurred between ATV and β-CD in the physical mixture and inclusion complex.

3.4. Differential Scanning Calorimetry studies

DSC runs are reported in Fig. 6. In the case of ATV (Fig. 6), a single sharp endothermic peak corresponding to the melting of the drug was observed (T_{onset}=154.09°C, T_{peak}=160.24°C, and ΔH=18.36 J/g). The DSC curves for each carrier have a single endothermic peak for the melting of PEG 4000 (63.39°C), PEG 6000 (66.90°C), D-mannitol (177.43°C), and PVP K30 (98.03°C).

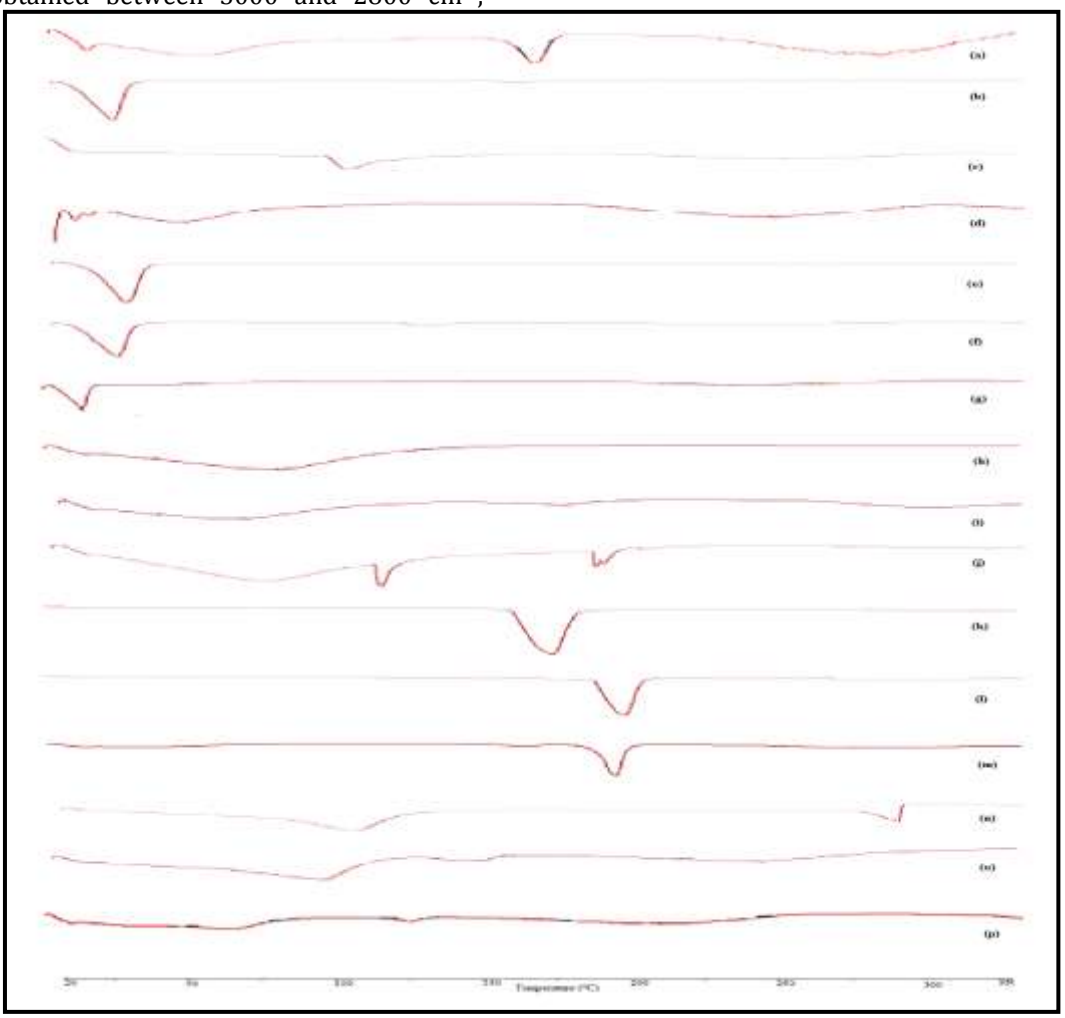

Figure 6: DSC thermogram of a) pure ATV; b) PEG 4000; c) ATV/PEG 4000 physical mixture; d) ATV/PEG 4000 solid dispersion; e) PEG 6000; f) ATV/PEG 6000 physical mixture; g) ATV/PEG 6000 solid dispersion; h) PVP K30; i) ATV/PVP K30 physical mixture; j) ATV/PVP K30 solid dispersion; k) D-mannitol; l) ATV/D-mannitol physical mixture; m) ATV/D-mannitol solid dispersion; n) β-CD; o) ATV/β-CD physical mixture; p) ATV/β-CD inclusion complex.

The DSC curves of β-cyclodextrin showed two endothermic peaks between 137.12°C and 304.30°C. The physical mixture and SD prepared by using PEG 4000 as carrier showed two endothermic peaks between 121.70°C, 229.95°C and 80.34°C, 233.19°C, respectively, demonstrating a marked shift in the endothermic peaks from original endothermic peaks of ATV and the PEG 4000. Also, a considerable decrease in peak height of the thermogram was observed. The endothermal peaks of physical mixture and SD indicate the possibility of formation of crystals of the solid dispersion; however, the marked decrease in height of the peaks and significant shift in the endothermal temperature suggests the presence of a significant proportion of the physical mixture and SD in amorphous form. The physical mixture and SD prepared by PEG 6000 showed a sharp endothermic peak at 64.89°C and 57.86°C, respectively. The disappearance of the endothermic peak of ATV demonstrates that ATV could be dispersed homogenously in the amorphous state and that no ATV crystallizes out of the dispersion. The lower the ΔH value (176.60 J/g for physical mixture and 42.29 J/g for SD), the more amorphous the product is, and this agrees with enhancement of the dissolution rate obtained. The physical mixture and SD prepared by D-mannitol also observed single endothermic peak at 172.29°C and 170.12°C. The disappearance of endothermic peak of ATV and lower the value of ΔH indicates that ATV could be present as more amorphous state than crystalline state, results in the enhancement of dissolution of ATV. The physical mixture and SD prepared by PVP K30 showed two endothermic peaks at 87.38°C, 156.80°C and 93.41°C, 164.75°C, respectively. The peaks are related to the peaks present in pure ATV and PVP K30. The thermogram of physical mixture and SD prepared by PVP K30 portrait less intense peaks than the pure ATV, indicating the presence of more amorphous state of ATV than crystalline could be dispersed in the physical mixture and SD. Three endothermic peaks were observed in the physical mixture (at 119.80°C, 162.66°C, and 233.32°C) and inclusion complex (at 105.68°C, 159.24°C, and 236.40°C) prepared by using β-cyclodextrin. In the physical mixture and inclusion complex, the endothermic peak of ATV is present. However, the reduction in endothermic peaks and significant shifts in the endotherm peaks might be due to partial conversion of crystalline form of ATV to amorphous form. The thermogram showed no evidence of the formation of solid complex or any chemical interaction between drug and carrier.

3.5. X-Ray Diffractometry Studies

X-ray diffraction analysis can be used to evaluate any changes in crystallinity of the drug when formulated into SD or inclusion complex. ATV is a crystalline drug and it gives characteristic peaks. Thus, XRD could be used to study any changes in crystallinity of the drug in an amorphous form, which could be one of the mechanisms responsible for improved dissolution [38]. Fig. 7 shows the diffractograms of ATV, different physical mixture, SDs, and inclusion complex prepared by different carrier and β-CD.

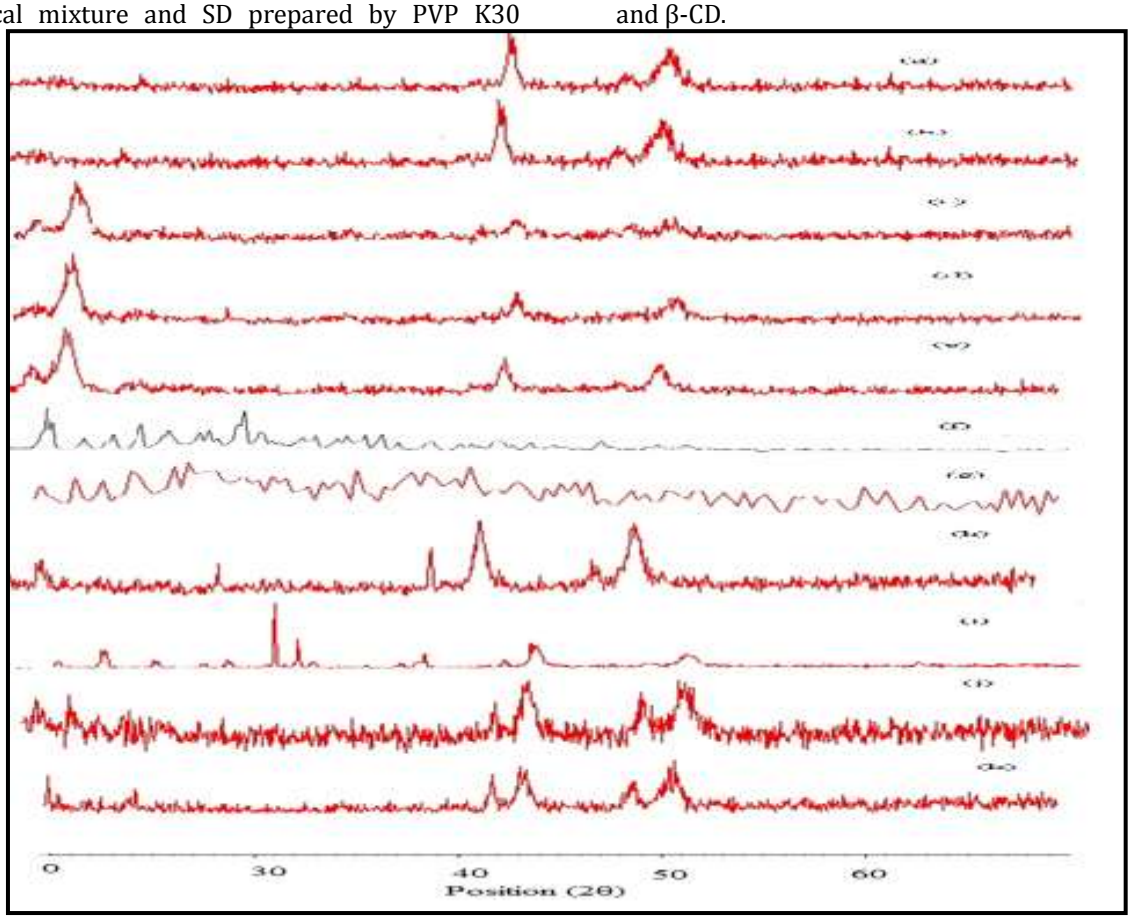

Figure 7: X-ray diffractogram of a) pure ATV; b) ATV/PEG 4000 physical mixture; c) ATV/PEG 4000 solid dispersion; d) ATV/PEG 6000 physical mixture; e) ATV/PEG 6000 solid dispersion; f) ATV/PVP K30 physical mixture; g) ATV/PVP K30 solid dispersion; h) ATV/D-mannitol physical mixture; i) ATV/D-mannitol solid dispersion; j) ATV/β-CD physical mixture; k) ATV/β-CD inclusion complex.

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The X-ray diffraction patterns of ATV reveals high crystallinity of the drug with major sharp diffraction peaks of high intensities and other peaks of lower intensities. A significant difference in the crystallinity was observed between physical mixture, SDs, and inclusion complex, prepared by different carriers as compared to pure ATV. In all the physical mixtures, SDs, and inclusion complex, the decreased in the intensity of the peak was observed except in physical mixture prepared by using D-mannitol. It might be due to the superposition of the crystalline nature of D-mannitol and ATV. DSC thermogram supported the proposed crystalline changes. This reduction of crystallinity may explain the higher drug release profile by the different physical mixtures, SDs, and inclusion complex as compared to ATV. A gradual reduction of intensity of the ATV peaks can be observed in SDs and inclusion complex as compared to their physical mixture. In contrast with the dissolution profile, the dissolution of ATV was increased from SDs and inclusion complex compared to physical mixtures. However, a new diffraction peak for physical mixture prepared by PEG 6000 and SDs prepared by PEG 4000, PEG 6000, and D-mannitol was observed, which suggests the possibility of formation of small amounts of crystal. The x-ray pattern of inclusion complex appeared to be different regarding the superposition of the ATV and β-CD patterns. These results confirm the formation of a new solid, from which we have the inclusion complex of ATV inside the cyclodextrin cavity.

3.6. Physical Characterization of Tablets

The hardness, weight variation, thickness, friability, disintegration, and content uniformity are described in Table 3. According to USP standards, the weight variation tolerance for uncoated tablets must be 5% or less. It was found that weight variation of the tablets compiled with the USP standard. The friability obtained (<1%) confirmed the suitability of wet granulation technology. Good uniformity in drug content was found in two batches of tablets. Tablets prepared with β-CD had a disintegration time of 15 sec. The tablet formulation fulfilled USP requirements (<15 min). In comparison with conventional tablets formulated without β-CD, inclusion complex tablets clearly performed better and a significant enhancement in dissolution characteristics was observed (Fig. 8).

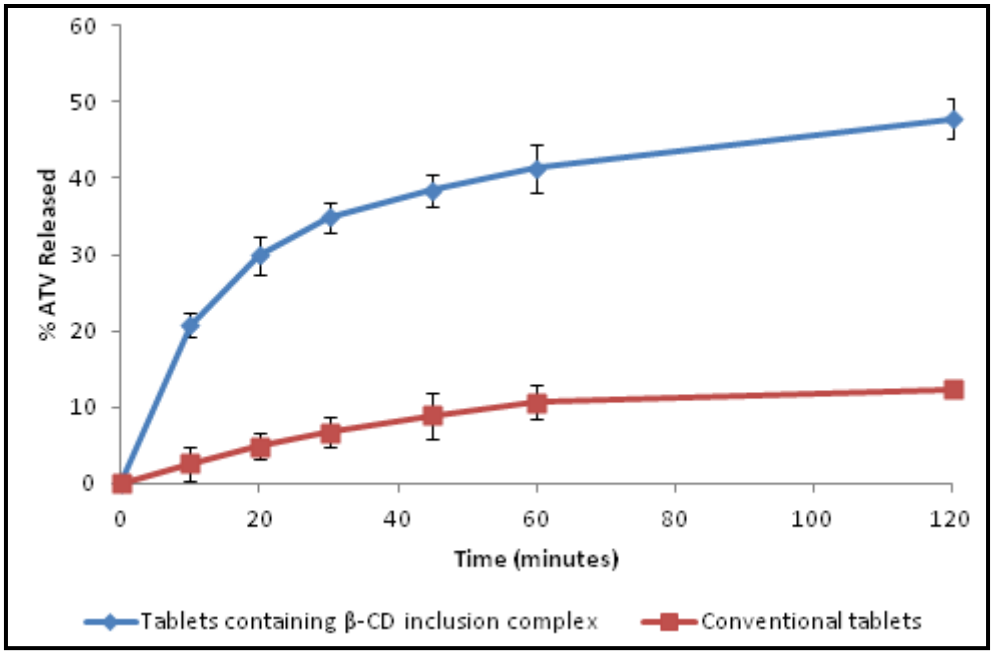

Figure 8: Dissolution profile of ATV from ATV conventional tablets and from ATV:β-CD at 1:3 drug:polymer ratio.

3.7. Pharmacokinetic Study

Based on dissolution performance tablets of ATV with β-CD in molar ratio of 1:3 (drug:polymer) was chosen for pharmacokinetic studies. The oral bioavailability of this formulation was compared with conventional tablets. Both formulations were orally administered in rabbits. Plasma samples were taken at different time, and drug concentration was determined by HPLC. Mean plasma concentration time results are shown in Fig. 9. The following bioavailability parameters were determined: t_{max}, C_{max}, and $AUC_{0\text{-}12hr}$; they are shown in Table 4.

Table 4: Pharmacokinetic data of conventional tablets and tablets containing inclusion complex (ATV and β-CD ratio 1:3).

	C_{max} (ng mL^{-1})	t_{max} (h)	AUC0-∞ (ng.h mL^{-1})	K_{el} (h^{-1})	$t_{1/2}$ (h)	$AUMC_{0\text{-}inf}$ (h^2.ng mL^{-1})	MRT (h)	Ka (h^{-1})
Conv. Tablet[a]	17005.67±368.41	0.5	226313.58±2469.10	0.047±0.01	15.08±2.51	1942768.69±58681.08	8.58±0.26	0.11±0.01
Tablet[b]	18229.18±354.49	0.5	247346.75±12219.30	0.047±0.01	14.73±1.40	2052687.22±116965.72	8.30±0.47	0.11±0.01

[a]conventional tablets containing no inclusion complex; [b]tablets containing inclusion complex

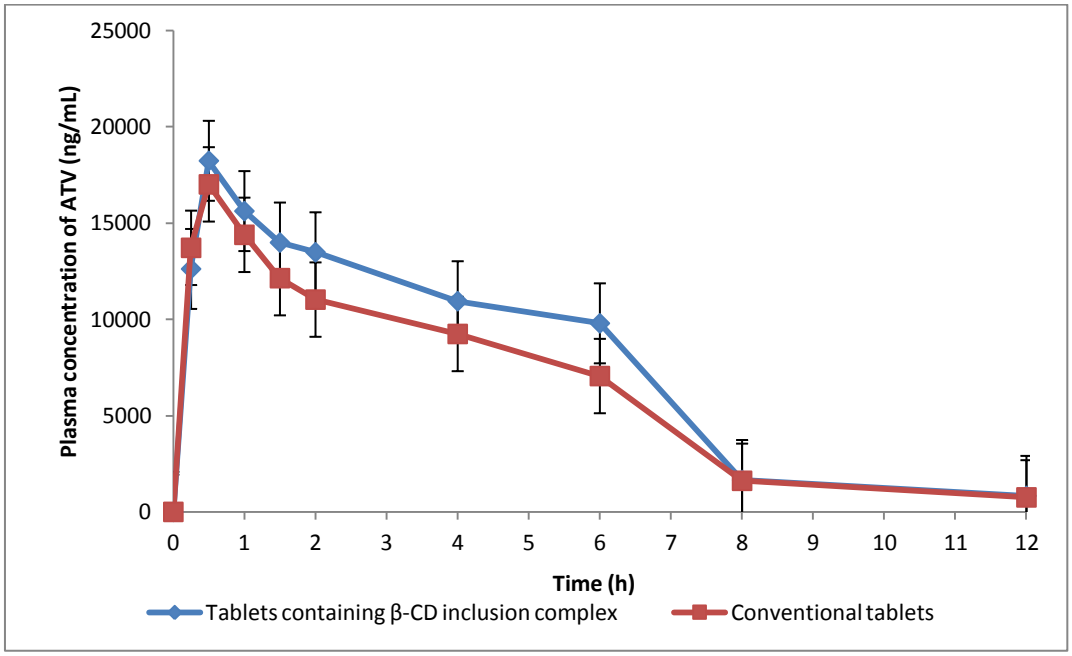

Figure 9: Mean ATV plasma concentration following oral administration of ATV alone and ATV/β-CD inclusion complex (Mean ± SE, n=3).

Fig. 9 shows the mean plasma concentration of ATV in rabbits after oral administration of tablets containing inclusion complex, compared with the plasma concentration given by conventional tablets. The peak plasma concentration of ATV from conventional tablet was found 17005.67±368.41 ng.mL^{-1} (C_{max}), which was lower than that of the tablets containing inclusion complex (18229.18±354.49 ng.mL^{-1}). There is an increase by 1.07 fold in C_{max} in case of tablet containing inclusion complex. The t_{max} was found similar in both the tablet formulations. The $AUC_{0-\infty}$ of conventional tablets and tablets containing inclusion complex was found to be 226313.58±2469.10 ng.hr mL^{-1} and 247346.75±12219.30 ng.hr mL^{-1}, respectively, which was 1.09 times greater than the tablet containing plain ATV as shown in Table 4. The pharmacokinetic analysis of the plasma level data confirmed that tablet containing inclusion complex have higher bioavailability than the drug itself, which could be attributed to the increase in solubility and dissolution rate of the drug. Stella and Rajewski also reported the use of β-CDs in oral formulations is to increased rate and extent of drug dissolution [39]. There are several reports showing that the aqueous solubility and dissolution rate of poorly soluble drugs are significantly increased in vitro by cyclodextrin complexation [40-42]. The present finding demonstrates a significant improvement in bioavailability of ATV by oral administration of its inclusion complex with β-CD in rabbits, owing to faster t_{max} and higher C_{max}. Inclusion complexation of poorly soluble drugs with β-CD may be expected to provide better bioavailability because of greater dissolution rates of drugs.

4.0. CONCLUSION

From the above analysis, it can be concluded that improved drug dissolution could be achieved by formulating ATV as solid dispersions with PEG 4000, PEG 6000, PVP K30, and D-mannitol and inclusion complex with β-CD. The solid dispersions and inclusion complexes showed higher saturation solubility with an increased rate of dissolution as compared with the physical mixtures of the drug. Tablets containing those inclusion complexes had drug dissolution profiles that were better than those of conventional tablets without β-CD. The physical properties of the tablets indicated that β-CD is suitable excipients for the development of ATV fast-release tablets. The oral bioavailability of ATV was able to improve through administration of tablets containing β-CD inclusion complex compared to tablets without β-CD.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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