Research Article

Effect of hydrophilic polymer on solubility and dissolution of Atorvastatin inclusion complex

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ABSTRACT

The present study was design to study solubility properties of inclusion complexes of atorvastatin, with *β*-cyclodextrin (βCD) and hydroxypropyl-*β*-cyclodextrin (HPβCD) and to analyze the effect of hydrophilic polymer on complexation, aqueous solubility and dissolution of drug. The phase solubility curves were classified as an A_L type for both binary and ternary systems showed that atorvastatin solubility increased linearly as a function of βCD concentration indicated formation of the inclusion complex. The molecular behaviors of atorvastatin in various samples were characterized by Fourier transform infrared spectroscopy, differential scanning calorimetry, and powder X-ray diffraction studies. Studies confirmed the transformation of crystalline atorvastatin to amorphous, best results obtained by combined effect of HPβCD and hydrophilic polymer in common solvent evaporation technique. The highest improvement in solubility, drug content were observed in inclusion complex prepared with HP*β*CD and polymer by common solvent evaporation method. The findings confirmes the addition of small amounts of hydrophilic polymers improves solubilizing and complexing ability of cyclodextrin which further related to increased release of drug in dissolution medium. This study signifies the use of hydrophilic polymers in combination with HPβCD for the formation of inclusion complex of atorvastatin.

Keywords: Inclusion complex, Atorvastatin, Hydrophilic polymers, Solubility, Cyclodextrin.

INTRODUCTION

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. Several methods have been proposed and used to improve the bioavailability of such drugs including micronization. [1,2] salt formation [3,4]; use of metastable polymorphs; solvent disposition; selective adsorption on insoluble carriers; solid dispersion [5,6] ; complexation with cyclodextrins[7] and solute solvent complexation. Cyclodextrin (CDs) are potential carriers for achieving such objectives. 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.[8] 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. [9-11] These strategies decrease the amount of CDs needed in solid oral dosage forms.[12]

Generally, CDs inclusion complexes are prepared by precipitating from saturated aqueous solution[13], kneading, freeze drying, and spray drying and using supercritical fluid. Present investigation was undertaken to enhance the aqueous solubility and dissolution rate of atorvastatin through formation of an inclusion complex with βCD, HPβCD and to investigate the effect of water-soluble polymers on the complexing abilities and aqueous solubility of CDs. Atorvastatin (ATV) comes under class II of biopharmaceutical classification system having absolute bioavailability of 14%.[14] ATV (R-(R*, R*)]-2-(4-fluorophenyl)-beta, delta-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenylamino) carbonyl] -1H -pyrrole-1-heptanoic acid) reduces total cholesterol, LDL –cholesterol in patients with homozygous familial hypercholesteremia, non- familial hypercholesteremia and mixed dyslipidemia.[15,16]

MATERIALS AND METHODS

MATERIALS

Atorvastatin calcium was procured from VAMA Pharma Ltd. (Nagpur, India). βCDs were obtained from S.D. Fine-Chem. Ltd. (Mumbai, India) and HPβCD of molecular weight 1400 from Glenmark Generics Ltd. (Mumbai, India). Polyvinyl pyrollidone k-30(PVP k-30) was obtained from Watson Pharma (Mumbai, India). Polyehylene glycol (PEG) 4000 and PEG-6000 was gifted by Loba Chemie Pvt. Ltd. (Mumbai, India) and Qualigens Fine Chemicals (Navi Mumbai, India) respectively. Other reagents and solvents used were of analytical reagent grade.

METHODS

Phase Solubility Studies:

Effect of hydrophilic polymer on solubility of ATV

Phase solubility studies were performed (Higuchi and Connors method)[17] by adding an excessive amount of ATV to aqueous solution containing increasing amount of various polymers (0-2.5% w/v).

Phase solubility studies for binary and ternary systems

Phase solubility studies were carried out (Higuchi and Connors method) by adding an excessive amount of ATV to aqueous solution containing increasing amount of CD (0-1.8% w/v, to its saturation solubility), HPβCD (0-1.8% w/v) and polymers (0-2.5% w/v).

For ternary systems same study was performed as that of binary but in presence of fixed amount of third component i.e. polymer (2% w/v). The mixtures were stirred for 24 hr at 30±2^{0}C, filtered and analyzed by spectrophotometer (Shimadzu 160A UV/VIS spectrophotometer, Shimadzu Corp, Tokyo, Japan) at 246 nm. The apparent stability constant was calculated from the initial straight portion (intrinsic solubility of ATV) of the phase solubility diagram using the following equation.

$$K_s = \frac{\text{Slope}}{S_0\,(1\text{-Slope})}$$

Where, S_0 is the solubility of ATV in absence of CDs.

Preparation of ATV binary and ternary complexes

Various procedures were used to obtain binary and ternary complexes of ATV. For binary product ATV was complexed with HPβCD in the ratio1:4. For ternary product ATV was complexed with HPβCD and PVP-K30 in a ratio 1:4:2.

Physical mixture (PM)

A physical mixture of ATV with HPβCD was prepared by mixing equimolar quantities homogenously in a mortar for one hour. For ternary system, physical mixture of ATV:HPβCD:PVP K30 was taken in ratio 1:4:2 and mixed in geometric proportion for one hour. All physical

mixtures further passed through sieve # 80 with minimum abrasion.

Kneading method (KP)

Binary and ternary physical mixtures were kneaded vigorously for 1 hour using small volume of water: methanol (1:1 v/v) solution to obtain homogeneous dispersion. Products then dried in the oven at the temperature 40^0C for 24 hours. Dried complex was pulverized into fine state and passed through sieve # 80.

Co –Solvent Evaporation method (CoS)

For binary product ATV was dissolved in methanol and HPβCD was dissolved in water to get clear solutions. The two solutions were then mixed with constant stirring on the magnetic stirrer. Resultant solution was then evaporated at 45^0C temperature for 24 hours. Dried complex was pulverized into fine powder and passed through sieve # 80.

For ternary products, PVP K-30 was dissolved in methanol along with binary mixture, and processed similarly as mentioned above.

Common Solvent Evaporation Method (CSE)

ATV and HPβCD were dissolved in pure methanol to get a clear solution. The solution was evaporated overnight at room temperature. The complexes so prepared were passed through sieve # 80.

For ternary product, PVP K-30 was dissolved along with binary system to get clear solution, and processed similarly as mentioned above.

Percentage yield

The efficiency of the process is determined by the yield acquired from the process. It is calculated as,

% yield = Practical yield / Theoretical yield × 100

Solubility analysis of the Cyclodextrin complexes

Excess of prepared binary and ternary inclusion complexes were dispersed in the 25 ml of distilled water in a screw capped bottles to get a supersaturated solution, shaken continuously (rotary flask shaker) for 24 hours at ambient temperature until equilibrium was attained. A sample (2ml) was withdrawn, filtered, further diluted and analyzed by spectrophotometer at 246 nm.

Determination of drug content in complexes

The amount of drug present in a 10 mg equivalent amount of powder was determined by; dissolving the powder in 100 ml methanol, from that 1 ml of solution was diluted and assayed for drug content by spectrophotometer at λ max 246 nm.

Characterization of complexes

Differential Scanning calorimetry

DSC (DSC Q20 V24.4 Build 116, USIC, K.U. Dharward, India) studies was conducted by placing the weighed samples in sealed aluminum pans using liquid nitrogen as coolant. The samples were scanned at 10^0C/min from 40^0C to 300^0C. DSC thermograms of pure ATV, HPβ-CD, PVP K-30, binary and ternary complexes prepared by CSE method were determined**.**

X-ray diffractometry

Powder X-ray diffraction patterns of pure ATV, β-CD, HPβ-CD, PVPK-30, binary and ternary complexes prepared by CSE method were analyzed using powder X-ray diffractometer system equipped with Cu as anode material and a graphite monochromator using a voltage of 40kv and a current of 30mA. The diffractograms were recorded in the 2θ angle range 10^0 to 80^0 and process parameter was set as: scan step size of 0.020(2θ).

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of pure ATV, β-CD, HPβ-CD, PVPK-30, binary and ternary complexes were obtained using Shimadzu FTIR-8400 spectrophotometer. The powdered sample was thoroughly mixed with dry powdered potassium bromide. The blend was then compressed into transparent disc under high pressure using special dies.

In vitro release studies for binary and ternary complexes

Drug release profile of binary and ternary complexes were obtained by USP II dissolution apparatus (TDT-50, Electrolab, Mumbai, India) using distilled water (900 ml) as dissolution medium at 50 rpm (37 ± 0.5°C). The samples were withdrawn at fixed interval, filtered (pore size 0.22 µm), diluted suitably with dissolution medium and analyzed for ATV content spectrophotometrically at 246 nm.

Formulation studies

Tablets containing complexes (equivalent to 10 mg ATV) prepared by CSE method was formulated using various excipients and evaluated for various pre-compression studies. The blend was compressed on rotary press tablet machine using concave shape, 6mm punch. Further subjected to various post compression studies. Drug release profile from tablets was determined using 6.8 pH buffers as dissolution medium at 50 rpm (37 ± 0.5°C).[18]

RESULT AND DISCUSSION

Phase solubility studies

Effect of hydrophilic polymers on solubility of the drug

Equilibrium solubility studies were performed in aqueous solutions to determine the solubilizing effect of different polymers on ATV. All the examined polymers showed a solubilizing effect towards ATV, which could be ascribed to weak polymer drug interactions. In all the cases, the optimal polymer concentration was found to be 2% w/v as further addition of polymer showed no further increase in drug solubility (fig. 1). PVP K-30 showed the maximum increase in the solubility as compared to other polymers.

Effect of polymers on the stability constants of complexes

The Phase solubility diagrams of ATV in aqueous CDs (βCD and HPβCD) solutions in absence and in presence of hydrophilic polymer (2% PVP K-30) are shown in fig.2. They demonstrate A_L type[17] equilibrium phase solubility diagram for both binary and ternary systems, showing that ATV solubility increase linearly as a function of CDs concentration. The increment of ATV solubility seems to be related to the inclusion ability of the CDs molecules in water.

The values of stability constants of ATV binary and ternary complexes (Table 2) shows that the slopes in all cases were less than unity, thus confirming the formation of 1:1 complexes. The binding potential of HPβCD was higher than the parent CD because hydrophobicity around the cavity increases due to the presence of alkyl chains. Therefore, Ks values of ATV-HPβCD binary and ternary complexes were greater than those obtained with ATV-βCD complex. ATV solubility and binding potential is found to be more with HPβCD than βCD. Hence complexation study was further carried out with HPβCD in binary and ternary form.

Effect of Polymer- Cyclodextrin combination on drug solubility

When 2% w/v of water soluble polymers and cyclodextrin are present together in the solution, one achieves an extent of drug solubilisation greater than when they are used separately.

Addition of polymers could contribute to improvement of the complexation ability of cyclodextrins by establishing interactions such as hydrophobic bonds,

Vander Waals dispersion forces, or hydrogen bonds and/or promoting the release of high-energy water molecules present in their cavity. PVP K-30, which exhibited the highest solubilising effect in binary systems, likewise showed the largest enhancing effect on cyclodextrin ATV solubilisation as compared to other tested polymers, which is also evident by large increase in stability constant in Table 2.

Percentage yield

The percent yield for binary and ternary systems was found to be maximum by physical mixture method followed by CSE and then Co-evaporation method.

Solubility analysis of the Cyclodextrin complexes

The prepared binary and ternary cyclodextrin complexes with the different methods were studied for their solubility in distilled water at room temperature for the duration of 24 hours (fig. 3).

The solubility of ATV was found to be highest when prepared by common solvent evaporation method for both binary and ternary system. The solubility of pure ATV in distilled water was 0.002072µg/ml. PM of binary and ternary systems showed an increased in solubility up to 0.13 mg/ml and 0.088 mg/ml respectively. By CSE method, binary and ternary system shows 0.108 mg/ml and 0.330 m/ml respectively. Results indicate that HPβCD complex of ATV by CSE method had increased solubility of the drug; maximum increase in solubility was observed for ternary complex.

Drug content

The percentages of drug content present in the binary & ternary complexes are mentioned in table 6.

The drug content of various products ranges from 71.89% to 99.31%. The drug content for binary and ternary system was found to be more by kneading method and CSE method. By CSE method binary product showed 98.75% and ternary product showed 99.31% of drug content.

Characterization of complexes

Differential Scanning calorimetry studies

DSC curves of pure components and the respective binary and ternary products in the melting range of the drug are given in fig.4.

DSC thermogram of ATV showing (fig.4) two endothermic peaks one of which at 150.71^0C corresponding to the melting point of crystalline ATV and another at 67.11^0C due to loss of water or dehydration. The DSC curve of pure ATV indicates its crystalline anhydrous state. The DSC thermogram of HPβCD (fig.6) exhibited broad endothermic peak at 86-90^0C attributed to the evaporation of absorbed water. The DSC curve of PVP K-30 (fig.3) showed a broad endothermic peak in the range of 50-60^0C owing to the softening of the polymer. In binary system, the peak for ATV was markedly broader and reduced in intensity as a consequence of interactions between components and/or drug amorphization respectively. For ternary system, the endotherm was more reduced in intensity and shifted towards lower melting range, indicating the absence of crystalline drug.

These thermal studies were indicative of formation of inclusion complex in solid state. The disappearance of an endothermic peak might be attributed to an amorphous state and/or to an inclusion complexation. In binary and ternary inclusion complex (fig.4 and fig.5) no peaks related to ATV were seen. This indicated that ATV no longer present in the crystalline form and might have been converted into the amorphous form.

XRD (X-ray diffractometer) studies

The X-ray diffraction patterns of ATV, βCD, HPβCD, PVP K-30 and binary and ternary products are shown in fig.5. XRD studies confirmed the DSC results. The X-ray diffraction patterns of ATV showed

sharp, highly intense and less diffused peaks indicating crystalline nature of the drug. Halo-patterns or define broad peaks were recorded for PVP K-30, HPβCD demonstrating their amorphous states. Moreover, for binary and ternary products patterns were apparently similar, showing the disappearance of crystalline peaks of ATV confirmed the transformation of crystalline ATV to amorphous due to combined effect of HPβCD and PVP K-30 in CSE technique. Many studies have reported that amorphous systems are efficient for the enhancement of dissolution and bioavailability.[19] In addition, amorphous systems offer 10–1600 folds of solubility enhancement than crystalline form.[20] Thus, the XRD studies demonstrated a significant reduction in the crystallinity of ATV indicating penetration of drug in HPβCD cavities.

Fourier transforms infrared spectroscopy (FTIR)

The FTIR studies of ATV, HPβCD, PVP K-30 and binary and ternary products are shown in fig.6. The prominent peaks of ATV was observed in the region of 3363.62 cm^{-1} due to the (-OH stretching), a peak at 3245.97 cm^{-1} due to the N-H stretching and a peak at 1650 cm^{-1} observed due to the carbonyl group. At the lower frequencies 1317 cm^{-1} (C-N stretching), 1215 cm^{-1} (C-O stretching) 1159 cm^{-1} for (C-F stretching) were observed.

Spectra of HPβCD shows the prominent peaks in the region of 3412 cm^{-1} due to the (-OH stretching), 2928 cm^{-1}(C-H stretching), 1645 cm^{-1}(H-O-H bending) and 1030 cm^{-1}(c-o-c stretching), respectively.

In case of complexes prepared using HPβCD, PVP K-30 showed considerable differences such as overlapping of O-H and N-H group peak resulting broadening of the peak. These modifications clearly indicated the presence of host guest interaction suggesting the formation of stable hydrogen bonds between ATV and cyclodextrins. Significant changes were recorded in IR spectrum of inclusion complexes. The decrease in frequency of specific peak is generally seen on complexation, which indicated an ordering of the molecule.

In vitro release studies

The ratio of polymer (PVP K-30) in the ternary complex was optimized based on the results from dissolution studies. A ratio of 1:4:2 (drug: HPβCD: PVP) was found to be the best combination as evident from the drug release studies (fig.7, fig.8 and fig.9). Drug release for both the systems was found to be more when prepared by common solvent evaporation method.

Formulation studies

The binary and ternary complexes prepared by CSE method were studied for physical properties to judge its tableting suitability generally, compressibility index values up to 15% and angle of repose between 25^0 and 30^0 often shows good to excellent flow property. % compressibility, angle of repose and physical prosperities of complexes are given in Table 7. The values found were according to the given limit indicating their suitability for tableting purpose. Prepared tablets also showed the values of friability, hardness, weight variation and drug content in an acceptable range (Table 8).

In vitro dissolution of various formulations at different time interval is reported in the (fig. 10). The tablets containing binary and ternary complexes showed faster and reproducible release as compared to the marketed preparations of ATV. Tablets containing ternary complex showed 69.81% drug release, whereas the marketed formulation showed only 21.29% of drug release in same time period. The results clearly indicate the advantage of improved aqueous solubility of ATV in complex form by HPβCD and PVP K-30; can be formulated as tablets with a better dissolution pattern.

CONCLUSION

On the basis of the physicochemical characterization techniques described in this work, the complex formation between ATV, CDs and water-soluble polymer (PVP K-30) was confirmed. Solubility studies showed linear increase in aqueous solubility of ATV with increase in concentration of βCD and HPβCD. HPβCD proved to have better solubilizing and complexing properties for ATV than the parent βCD, as could be stated by the higher Ks values obtained for both binary and ternary complexes. The greater Ks values found for ternary complexes in comparison with the corresponding binary ones suggest a significant improvement on the complexation efficiency between ATV, HPβCD by addition of small amounts of water soluble polymers. The addition of hydrophilic polymers resulted in higher complexation efficiency and markedly enhanced the solubilizing efficiency of HPβCD. In vitro studies in distilled water for inclusion complexes of HPβCD with hydrophilic polymers showed increase in rates of dissolution several times higher than those of ATV and its complexes with HPβCD alone. The finding confirmes the addition of small amounts of hydrophilic polymers improves solubilizing and complexing ability of cyclodextrin which further related to increased release of drug in dissolution medium. Study signifies the use of hydrophilic polymers in combination with HPβCD for the formation of inclusion complex of ATV.

Table I: Formulation of tablets using common solvent evaporation cyclodextrin complexes

S.No.	Ingredients	F1 (mg) (Binary complex)	F2 (mg) (Ternary complex)
1	ATV Complexes	70	50
2	Avicel	50	50
3	Lactose	60	60
4	Aerosil	5	5
5	Magnesium stearate	5	5
	Total (mg)	190	190

Tablets are prepared in batch of 40.

Table II. Stability constants (Ks) for binary and ternary systems of ATV with Cyclodextrins

System	r^2	Slope	S	K_s	K_{TS}/K_B
Drug-βCD	0.9797	0.1283	0.002072	53.98	---
Drug-HPβCD	0.9822	0.5028	0.002072	120.65	---
Drug-HPβCD-2%w/vPVP K-30	0.9913	0.9587	0.002072	272.57	2.26

K_{TS}/K_{BS} is the ratio of Ks for ternary and binary complexes.
S= Solubility of ATV in aqueous solution.
K_{TS}/K_{BS} is the ratio of Ks for ternary and binary complexes.
S= Solubility of ATV in aqueous solution.

Table III: Effect of polymers on solubilization of ATV in aqueous HPβCD solution

Polymer	S_1 (µg/ml)	S_2 (µg/ml)	S_2/S_0
PEG 4000	8.7444	14.5686	1.45
PEG 6000	9.5601	13.9412	1.39
PVP K-30	12.5902	14.6471	1.47

S_1=Solubility of ATV in aqueous solution containing 2% w/v polymer.
S_2=Solubility of ATV in aqueous solution containing 2% w/v polymer & 1.8%w/v HPβCD.
S_0= Solubility of ATV in aqueous solution containing 1.8% w/v HPβCD (10 µg/ml).
S_2/S_0=Solubility ratio.

Table IV: Percentage yield of binary complexes by different methods

Sr. No	Binary complex	Theoretical yield (g)	Practical yield (g)	% yield
1	Physical mixture	2.5	2.381	95.24
2	Kneading method	2.5	1.927	77.08
3	Co-evaporation method	2.5	2.032	81.21
4	Common solvent method	3.5	3.096	88.46

Table V: Percentage yield of ternary complexes by different methods

Sr. No	Ternary complex	Theoretical yield (g)	Practical yield (g)	% yield
1	Physical mixture	2.5	2.406	96.24
2	Kneading method	2.5	1.977	79.08
3	Co-evaporation method	2.5	2.132	85.28
4	Common solvent method	3.5	3.146	89.88

Table VI: Amount of drug present in the prepared binary inclusion complexes

S. No	Method of Preparation	Drug Content	
		Binary complex (1:4)	Ternary complex (1:4:2)
1	Physical mixture	71.89 ±0.275	86.44±0.291
2	Kneading method	98.33 ±0.275	94.07±0.289
3	Co-evaporation method	84.44±0.135	89.77±0.080
4	Common solvent method	98.75±0.140	99.31±0.271

Mean ± SD; n=3

Table VII: Pre-compression parameters for evaluation of prepared tablet formulations

Formulation	Tapped Density (g/ml)*	Untapped Bulk Density (g/ml)*	Compressibility (%)*	Angle of Repose (^{0})* Code
F1	0.5128±0.010	0.444±0.0157	13.42±0.0173	25.99±0.0772
F2	0.5000±0.0058	0.4255±0.0055	14.90±0.0115	26.78±0.9873

Mean ± S.D. * *n=3*

Table VIII: Post-compression parameters for evaluation of prepared tablet formulations

Formulation Code	Friability* (%)	Hardness* (Kg/cm^2)	Thickness* (mm)	Diameter* (mm)	Weight variation# (g)	Drug Content#(%)
F1	0.310±0.074	4.43±0.187	6.52±0.242	6.13±0.100	0.189±0.003	99.62
F2	0.327±0.156	4.27±0.312	6.45±0.344	6.16±0.065	0.187±0.003	97.92

Mean ± S.D. * *n=6*, # *n=20*

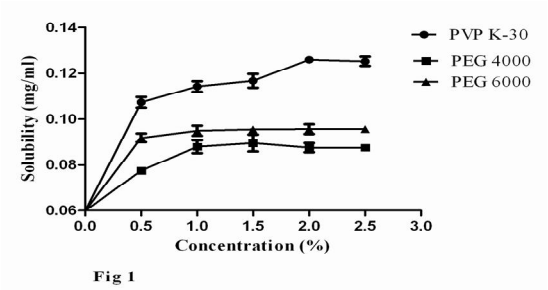

Fig. 1: Phase solubility curve of various drug: polymer binary systems

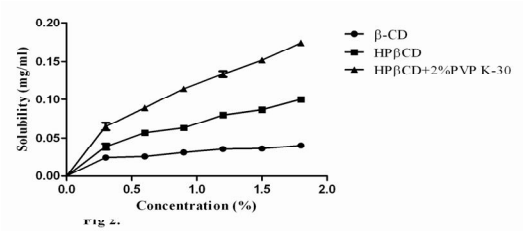

Fig. 2: Phase solubility diagram of ATV in presence of βCD (-♦-) and HPβCD without water soluble polymers (-▪-) & with 2% (w/v) PVP (-▲-)

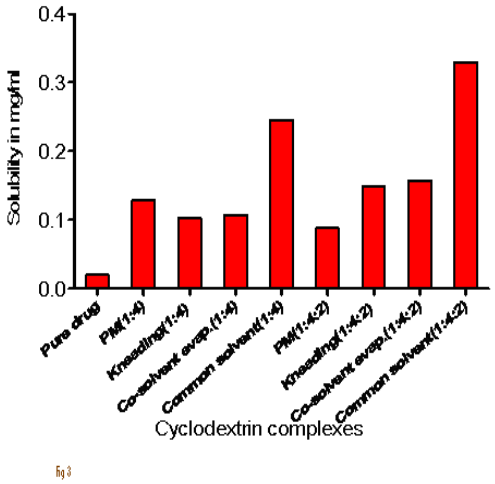

Fig. 3: Solubility study profile of various cyclodextrin complexes in distilled water

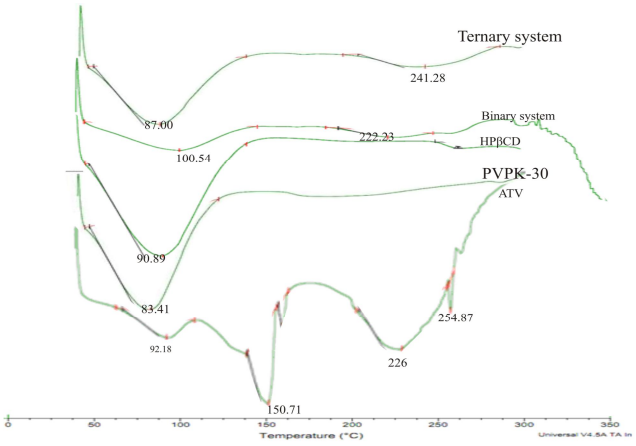

Fig 4

Fig. 4: DSC thermogram of Atorvastatin, PVP K-30, HPβCD, binary complex and ternary complex prepared by common solvent evaporation method

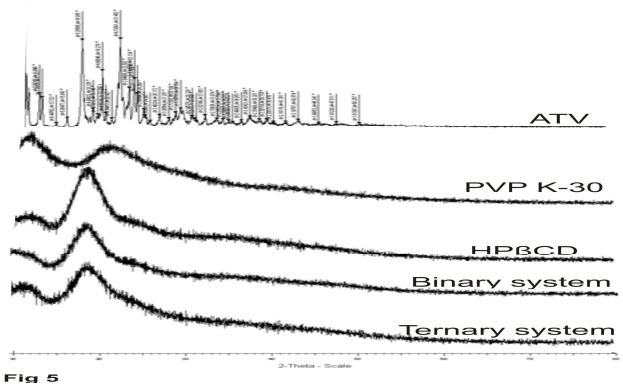

Fig 5

Fig. 5:X-ray diffraction patterns of Atorvastatin, HPβCD, PVP K-30, binary and ternary complexes prepared by common solvent evaporation

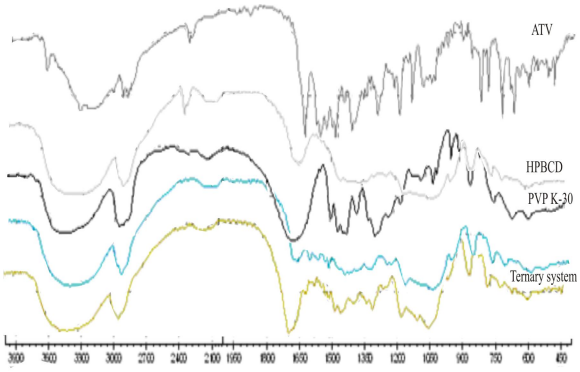

Fig 6

Fig. 6: FTIR Spectrum of Atorvastatin, HPβCD, PVP K-30, binary and ternary complexes prepared by common solvent evaporation

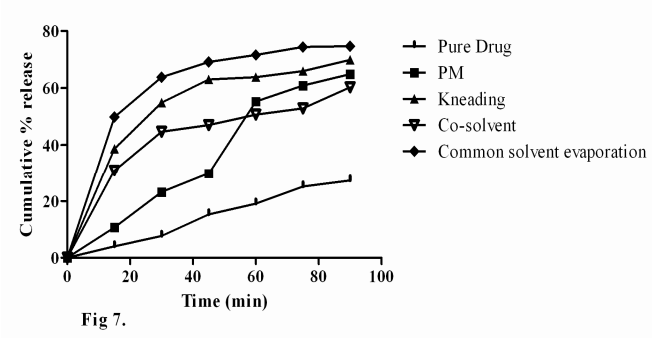

Fig 7.

Fig. 7: In- vitro release studies of binary cyclodextrin complexes

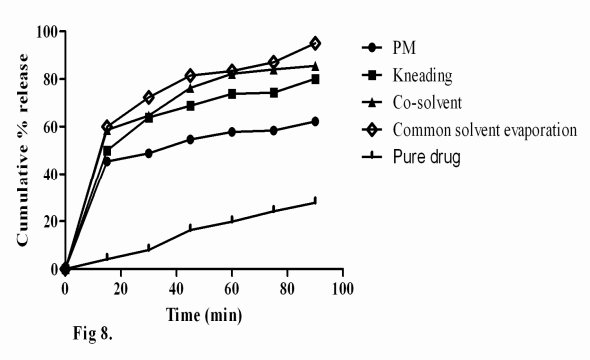

Fig. 8: *In-vitro* release studies of ternary cyclodextrin complexes

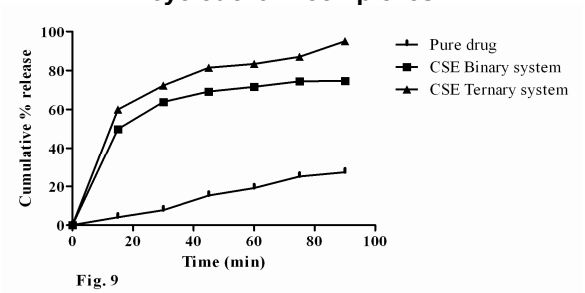

Fig. 9: *In vitro* release studies of cyclodextrin complexes

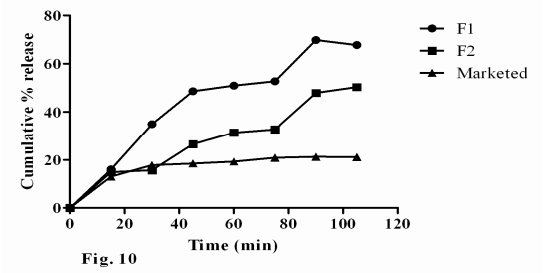

Fig. 10: Comparison of *in- vitro* dissolution studies of formulation with marketed tablet

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