



Inclusion complexes of atorvastatin calcium–sulfobutyl ether β cyclodextrin with enhanced hypolipidemic activity

Anureet Arora¹, Geeta Aggarwal², Thakur Gurjeet Singh¹, Manjinder Singh¹, Gitika Arora³, Manju Nagpal^{1*}

¹Chitkara College of Pharmacy, Chitkara University, Punjab, India.

²Delhi Pharmaceutical Sciences and Research University, Govt. of NCT of Delhi, New Delhi, India.

³NCRDs Sterling Institute of Pharmacy, Navi Mumbai, India.

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ABSTRACT

The aim of present study was to explore the impact of Atorvastatin (ATR) Sulfobutylether beta-cyclodextrin complex (ATR-SBE- β -CD) on ATR dissolution behavior. Various batches of inclusion complexes were formulated using various drug: polymer ratios (1:1, 1:3, and 1:5); using β -CD and SBE- β -CD and using two methods (freeze drying and kneading method). Phase solubility studies were carried out of all the complexes and ratio 1:5 (ATR-SBE- β -CD) prepared by freeze-drying yield maximum solubility enhancement (30-fold in comparison to pure drug). Fourier transformation infrared spectroscopy, Powder X-ray diffraction, Scanning electron microscopy (SEM), Differential scanning calorimetry (DSC) studies was also carried out. FTIR studies showed no drug polymer interaction. DSC and SEM studies suggested incorporation of drug into inclusion complexes of cyclodextrin. Solid dispersion via Freeze drying technique using SBE- β -CD (1:3 ratio of drug to polymer) produces better dissolution characteristics in comparison to kneading method. The results revealed superiority of SBE- β -CD over β -CD for solubility enhancement of poorly soluble drugs (owing to amorphous nature and more stable form of SBE β -CD). No significant drug loss was observed in solid dispersion batch (as per results of drug content analysis) during storage for 3 months under accelerated conditions. Further *in vivo* pharmacodynamics studies of selected batch were carried out by inducing obesity in rats by feeding them with a high-fat diet. Group I (normal control group) received normal chow diet and group II, group III, group IV (High fat diet group, optimized formulation group and disease control group) received HFD for 1 month and were further evaluated for BMI, Blood glucose, lipid profile, liver profile, and histopathological examination. The results so obtained depicted that optimized formulation of Atorvastatin (10 mg/kg, p.o.) showed better results in comparison to pure Atorvastatin Calcium (10 mg/kg, p.o.).

INTRODUCTION

Atorvastatin calcium (ATR) is one of the most favored statins, used to treat moderate-to-severe hypercholesterolemia. It leads to decrease in levels of low-density lipoproteins (LDL), i.e., bad cholesterol and triglycerides and simultaneously increases high-density lipoproteins level (HDL), which is the good cholesterol. This successful molecule was introduced by Pfizer Inc., USA as Lipitor[®]. However, the low aqueous solubility

of drug (i.e., 0.1 mg/ml; BCS class II drug), incomplete intestinal absorption, hepatic first pass metabolism, and crystalline nature makes it poorly bioavailable (only 12%). ATR is unstable to light, heat, and moisture. Due to this, the hydroxyl acid form of ATR is changed to the lactone form. The hydroxyl acid form act as a free acid moiety and it exhibits 15 times more solubility than lactone form due to the ionization of hydroxyl acid. The difference in physicochemical properties (Log *p* value 1.53 for hydroxyl acid and 4.2 of lactone at pH of 7.4) of the hydroxyl acid and lactone of ATR affects their biologic and therapeutic performance. ATR can further be destabilized by excipients used in the development of formulation (Khan *et al.*, 2011). Thus, this preferred statin has a good therapeutic value, but absorption is limited to biological system.

*Corresponding Author
Manju Nagpal, Chitkara College of Pharmacy, Chitkara University,
Patiala, India. E-mail: nagpalmanju@gmail.com,
manju.nagpal@chitkarauniversity.edu.in

Poor performance of ATR in biological system leads to need of large doses resulting in liver abnormality, kidney failure, arthralgia, rhabdomyolysis, and other adverse effects (Choudhary *et al.*, 2012). There is the unmet need to develop more bioavailable and stabilized formulation of poorly soluble drug ATR.

Enhancement of solubility, stability and *vis-a-vis* bioavailability (BA) of a poorly soluble drug is a challenging job for a drug formulation scientist. Most of the new chemical entities have good therapeutic impact, but their absorption is limited to the biological system due to poor solubility (Fazil *et al.*, 2016).

Many approaches have been tried by the researchers to enhance BA of ATR, which includes solid dispersions (Rodde *et al.*, 2014; Shaker, 2018), use of surfactants (Patel *et al.*, 2014), complexation with cyclodextrins, polymorphism, salt formation, size reduction, emulsification (Yeom *et al.*, 2016), gastroretentive formulation (Khan *et al.*, 2011), and nanotechnology (Zidan *et al.*, 2018) etc.

Choudhary *et al.* (2012) formulated solid dispersions of ATR employing skimmed milk as carrier, suggested as a promising approach for oral delivery of atorvastatin. To overcome the problem of instability and BA, various experiments were performed, which included ATR-cyclodextrin complexation. Zidan *et al.* (2018) explored the complexation of ATR with β -cyclodextrin (β -CD) based nanosponge carriers with improved dissolution characteristics and eventually the enhanced oral BA of ATR.

Cyclodextrins (CD) lead to improved BA of poorly aqueous soluble moieties by stabilization of drug molecules at the biomembrane surface. Moreover, use of CD leads to reduced gastrointestinal drug irritation, conversion of liquid drugs into microcrystalline form or in amorphous state and also overcome drug-drug and drug-excipient interactions etc. Palem *et al.* (2009) observed increase in stability, solubility and *in vitro* dissolution characteristics of ATR through inclusion complexation with β -CD.

The natural CDs are generally proven to be non-toxic for oral administration. However, γ -cyclodextrin tends to aggregate in aqueous solutions and therefore not preferred in parenteral formulations. The reported nephrotoxicity of β -CD limit their use in parenteral products. Lipophilic cyclodextrin derivatives, i.e., methylated cyclodextrins are reported to be toxic in parenteral formulations. Modified cyclodextrins are helpful in better fitting and association between cyclodextrin and entrapped drug, with simultaneous stabilization of the entrapped entity thereby reducing drug mobility and reactivity and also help in attachment of specific (catalytic) groups to the binding site.

Captisol, i.e., Sulphobutylether β -Cyclodextrin (SBE β -CD) is an exclusively modified form of cyclodextrin. The chemical structure is logically designed for maximum safety and optimized interaction with drug molecules for improving the stability, solubility and BA along with decreasing the volatility, irritation, any odor or taste. The solubility of Captisol in water is 70 g in 100 ml which is remarkably higher than β -cyclodextrin (i.e., 1.85 g in 100 ml). No nephrotoxicity and cytotoxicity is reported with captisol (Lankalapalli *et al.*, 2018).

Kulkarni *et al.* (2018) also demonstrated that Captisol inclusion complex is an effective strategy for solubility, stability and BA enhancement of asenapine. Ajmeera *et al.* (2015) indicated that captisol can be successfully employed as a potential carrier for

the oral delivery of telmisartan with enhanced BA and industrial applicability in terms of scale up.

Based on this hypothesis, the intent of current study was to explore the use of modified β -cyclodextrin i.e. captisol, for enhancing the oral BA of ATR by formulating its inclusion complex with captisol.

MATERIALS AND METHODS

Materials

Atorvastatin was kindly gifted by Mylan Labs, Hyderabad. Captisol (Sulfobutyl ether β -CD) was kindly gifted by Cydex Pharmaceuticals, Kanas; β -CD was procured from Sigma-Aldrich, India. All the other chemicals used in the study were of AR grade.

Methods

Formulation of inclusion complexes using β -CD and SBE β -CD

In current study, the inclusion complexes of atorvastatin were developed by using two approaches.

Kneading method: Accurately weighed the drug atorvastatin and polymers β -cyclodextrin and SBE β -cyclodextrin were taken in a ratio 1:1, 1:3, and 1:5 in the mortar and kneaded with small quantity of the 70% ethanol. The kneaded mixture was placed in hot air oven till a constant weight of dried product was obtained and sieved through 80 (#) mesh. The resulting mixture was preserved in the air tight container at room temperature.

Lyophilization method: Inclusion complexes were prepared via solvent evaporation method. Pure drug atorvastatin and the polymers, i.e., β -cyclodextrin and SBE β -cyclodextrin, were mixed in the ratios of 1:1, 1:3, and 1:5 in the 25 ml of solvent (water) in the beaker. The mixture was kept on magnetic stirrer for 2 hours. The mixtures were removed and cooled at room temperature and later kept for pre-freezing at a temperature of -20°C for 90 minutes. The pre-frozen sample was kept for freeze drying at a temperature of -70°C for 48 hours resulting in formation of fine powdered inclusion complexes.

Table 1 shows the composition of various inclusion complexes.

Table 1. Composition of inclusion complexes.

Formulation code	Drug (mg)	β -CD (mg)	SBE β -CD (mg)
KN1C*	100	--	100
KN3C	100	--	300
KN5C	100	--	500
KN1 β **	100	100	--
KN3 β	100	300	--
KN5 β	100	500	--
FD1C	100	--	100
FD3C	100	--	300
FD5C	100	--	500
FD1 β	100	100	--
FD3 β	100	300	--
FD5 β	100	500	--

*Represents SBE β -CD (Captisol) and ** represents β -CD.

Evaluation of inclusion complexes

Equilibrium solubility studies: The solubility of various inclusion complexes and ATR was determined in phosphate buffer of pH 6.8 at 37°C. The prepared formulations equivalent of 20 mg of drug was kept in covered conical flasks containing 25-ml phosphate buffer and further the dispersion was sonicated for 30 minutes at $37 \pm 0.5^\circ\text{C}$. After sonication, the flasks were kept in orbital shaker incubator at a temperature of $37 \pm 0.5^\circ\text{C}$ for 24 hours. The resulting solution was filtered and analyzed for drug spectrophotometrically (Systronics, India) at 244 nm.

Fourier transform infrared spectroscopy (FTIR): FTIR spectra of the samples were determined via potassium bromide pellet method using FTIR spectrophotometer (Spectrum 400, Perkin Elmer). Transparent pellets obtained were scanned over frequency range from 400 to $4,000\text{ cm}^{-1}$.

X-Ray diffraction: Powder X-Ray Diffraction patterns were traced of the samples using Ni filtered Cu (K- α) radiations and at a voltage of 45 kV and a current of 40 mA using (X'Pert Pro). The samples were scanned over 2θ range of $0-50^\circ$ with scan step size of 0.0170° (2θ) and scan step time of 25 seconds.

Differential scanning calorimetry: Thermal properties of atorvastatin calcium and inclusion complexes were studied via differential scanning calorimetric analysis (DSC 821^e, Mettler Toledo, USA). The samples were sealed in aluminium pan and scanned in temperature range from 30°C to 300°C and at a heating rate of $10^\circ\text{C}/\text{minute}$ in nitrogen atmosphere.

Scanning electron microscopy: External morphology of various samples was studied scanning electron microscopy (JSM 6100, JEOL). The samples were mounted onto the stubs using double-sided adhesive tape and which was coated with gold palladium alloy ($150-200\text{ \AA}$) using fine coat ion sputter.

In vitro dissolution studies: The *in vitro* release of ATR from various inclusion complexes was determined in 900-ml phosphate buffer of pH 6.8, kept at $37 \pm 0.5^\circ\text{C}$ and at 100 rpm using USP (Type II) dissolution apparatus (Electro Lab, India). 5 ml aliquots were withdrawn at specified intervals (5, 15, 30, 45, 60, 90, and 120 minutes), suitably diluted and analyzed spectrophotometrically for drug at 244 nm.

In vivo pharmacodynamics studies

Animals

Wistar male rats were employed in current study. The experimental protocol was suitably approved by Institutional Animal Ethics Committee (1181/PO/ReBi/S/08/CPCSEA). Animals (rats) were kept according to guidelines of CPCSEA, i.e., Committee for the Purpose of Control and Supervision of Experiments on Animals in Chitkara College of Pharmacy, Chitkara University Punjab, India. Rats were fed with normal chow diet (i.e., commercial rat pellets from Kisan Feeds Ltd. Mumbai) and *ad libitum* under controlled temperature and relative humidity (RH) of ($24^\circ\text{C}-28^\circ\text{C}$ and $60\%-70\%$, respectively) and natural light/dark cycle (12:12) animals.

High fat diet (HFD) induced obesity: Rats were kept on prepared HFD and water *ad libitum* for the 8 weeks time period. The experimental diet (g/kg) contents were as per the formula of Srinivasan *et al.* (2005).

Experimental protocol: 24 rats were used and divided into four groups of 06 rats each. Group I rats were maintained on standard chow diet for 08 weeks and water *ad libitum*, with no treatment. Group II rats were maintained on HFD for 8 weeks to persuade obesity. Group III rats received pure drug atorvastatin (10 mg/kg/day , *p.o*) for 4 weeks, started at the end of 4th week. Group IV rats received optimized test formulation (10 mg/kg/day , *p.o*) for 4 weeks, started at the end of 4th week.

Morphological parameters for obesity

Body weights of rats were measured once in a week.

Body mass index (BMI) was calculated from formula (Friedewald *et al.*, 1972; Krachler *et al.*, 2013):

$$\text{BMI} = \text{body weight (g)} / \text{length}^2 (\text{cm}^2).$$

Sample collection: Blood samples from overnight fasted rats were collected at the end of 8th week by retro-orbital puncture under ether anesthesia. The separated serum samples were stored at -20°C until used for further calculation of various biochemical parameters by enzymatic colorimetric methods using commercial kits.

Biochemical estimation

Estimation of HDL, LDL and total cholesterol (TC) was done by autoanalyzer using Bayer Diagnostic kit and triglycerides estimation was done by using Erba Diagnostics kit. Glucose-peroxidase method was used to calculate serum glucose levels.

Histopathological analysis: Separated liver samples from rats were fixed in buffered formalin (10% neutral), embedded in paraffin for histological examination. Standard liver tissue sections of 5 mm thickness were cut; stained with eosin and haematoxylin, and then examined under light microscope.

Statistical analysis: Results were expressed as mean \pm standard error of mean (SEM.). Data were analyzed using one-way analysis of variance followed by Bonferroni multiple comparison test as *post-hoc* analysis. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Equilibrium solubility studies

The solubility of the ATR in pH 6.8 phosphate buffer was found to be $54.1212\text{ }\mu\text{g/ml}$. Addition of cyclodextrins (β -CD and SBE β -CD) leads to increase in equilibrium solubility of drug. Captisol (i.e., SBE β -CD, modified form of cyclodextrins) based inclusion complexes showed significant increase in solubility of drug in comparison to inclusion complexes of drug using β -CD.

The effect of method of formation of inclusion complexes was also observed, i.e., freeze dried or lyophilized formulations showed dramatic increase in solubility of the drug in comparison to that of kneading method. Moreover, the yield of final formulations is less in kneading method and lyophilized batches yield almost $95\%-100\%$ final product. The results revealed that Captisol inclusion complexes of drug prepared by freeze drying method lead to maximum solubility of drug in 1:3 ratio of drug to Captisol. The comparative solubility data is shown in Figure 1.

Fourier transform infrared spectroscopy

FTIR spectrum of ATR showed characteristic peaks at $1,651\text{ cm}^{-1}$ (C=O stretch), $3,365\text{ cm}^{-1}$ (O-H stretch), $1,435\text{ cm}^{-1}$ (C-F stretch), $1,317\text{ cm}^{-1}$ (C-O stretch), $1,216\text{ cm}^{-1}$ (C-N stretch), and signal at 691.54 cm^{-1} is due to aromatic out of plane bend. FTIR spectrum of β -CD showed C-H stretching at $2,925\text{ cm}^{-1}$, N-H stretching at $3,307\text{ cm}^{-1}$, C-H bending at $1,417\text{ cm}^{-1}$, and C-Br stretching at 609 cm^{-1} . FTIR spectrum of SBE β -CD showed C-H stretching at $2,938\text{ cm}^{-1}$, C-O stretching at $1,412\text{ cm}^{-1}$ and C-N vibrations at $1,043\text{ cm}^{-1}$. FTIR spectra of various inclusion complexes (KN5 β , KN5C, FD5 β , and FD5C) showed that most of the characteristic peaks of drug are missing (signals at $3,365$, $1,317$, $1,216\text{ cm}^{-1}$) and some signals have shifted (e.g., $1,435$ to $1,420\text{ cm}^{-1}$) which is indicative of interaction of drug with both β -cyclodextrins (Figs. 2 and 3).

X-Ray diffraction

Atorvastatin calcium showed sharp peaks of the diffraction angle of 2θ at 9.2367 , 10.3544 , 11.9284 , 17.0248 , and 21.5218 . Diffraction patterns of β -CD showed various peaks at diffraction angle of 2θ at 12.4921 , 19.5868 , 22.7177 , 27.0674 , and 39.9618 , suggested crystalline nature of β -CD. In β -CD inclusion complexes (i.e., KN5 β and FD5 β), some of the peaks of drug are present but with reduced area and intensity and in addition peaks of β -CD was also observed (Fig. 4). The diffraction pattern of SBE β -CD with no sharp peaks, suggests amorphous nature of polymer. The absence of main peaks of drug or decreased area of some characteristic peaks of drug in inclusion complexes prepared by freeze drying and kneading (KN5 β ; FD5 β and KN5C; FD5C) indicated strong interaction of drug with β -CD and SBE β -CD and suggested formation of single amorphous complex (Fig. 5).

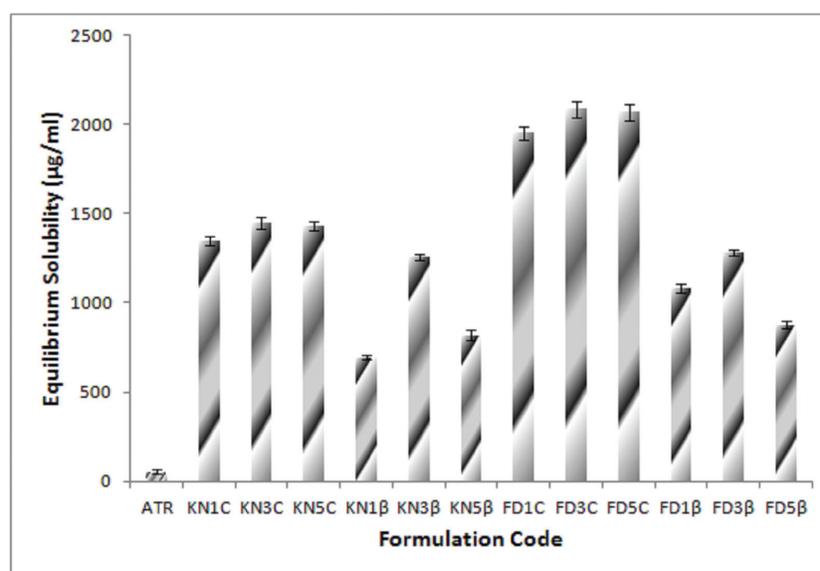


Figure 1. Equilibrium solubility studies of Atorvastatin Calcium inclusion complexes with β -CD and SBE β -CD.

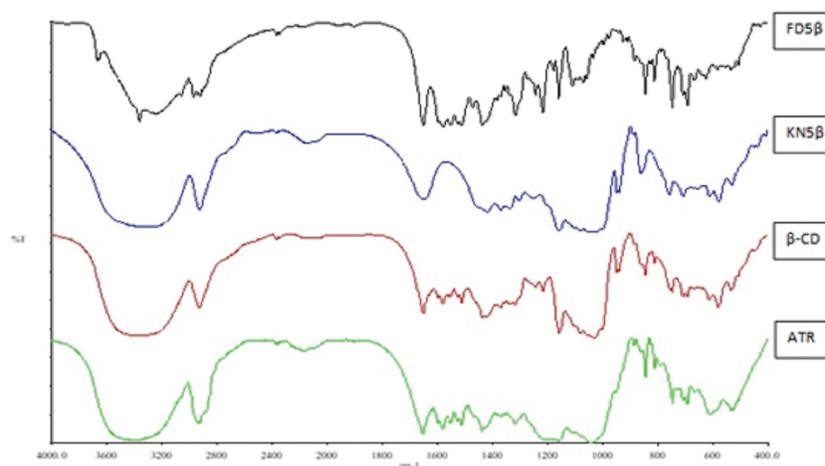


Figure 2. Overlay diagram of FTIR of ATR, β -CD, FD5 β , and KN3 β .

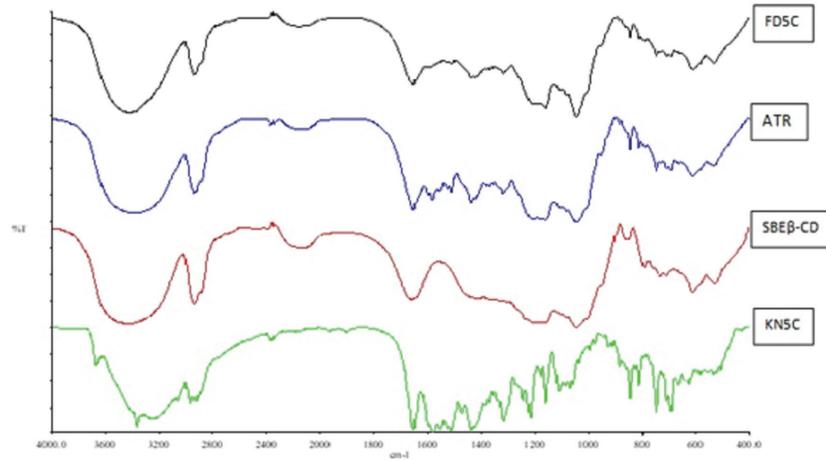


Figure 3. Overlay diagram of FTIR of KN5C, SBE β -CD, ATR, and FD5C.

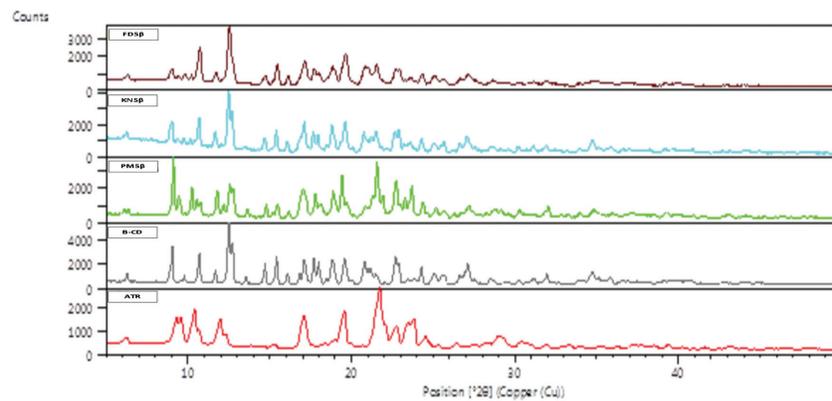


Figure 4. Overlay diagram of XRD of ATR, β -CD, PM5 β , KN5 β , and FD5 β .

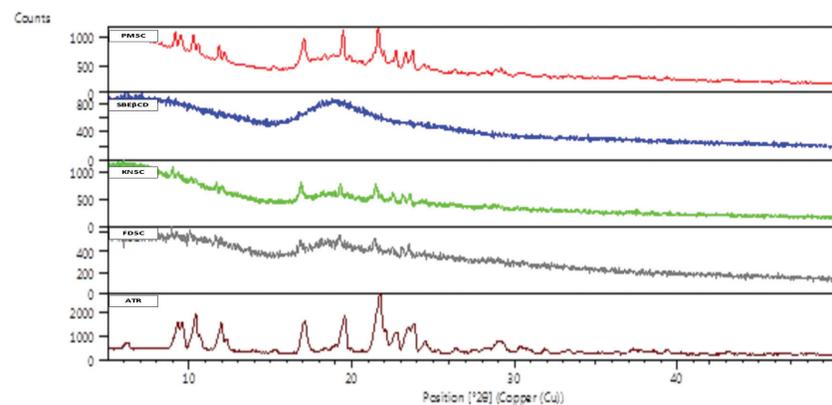


Figure 5. Overlay diagram of XRD of ATR, SBE β -CD, PM5C, KN5C, and FD5C.

Scanning electron microscopy

Scanning electron micrographs of pure drug ATR, SBE β CD and inclusion complex (FD5C and KN5C), are shown in Figure 6. The drug appeared as discrete particles rectangular needle shaped indicating its crystalline nature and the polymer (SBE β -CD) appeared as round, oblong in shape. SEM images of FD5C and KN5C inclusion complexes indicated changes in

topological characteristics of drug particles aggregated with each other, suggesting the amorphous nature of the product and existence of one component suggesting the maximum complexation.

Differential scanning calorimetry

DSC thermogram of ATR showed sharp endothermic peak at 165.41°C with enthalpy of fusion 80.873 J/g relating

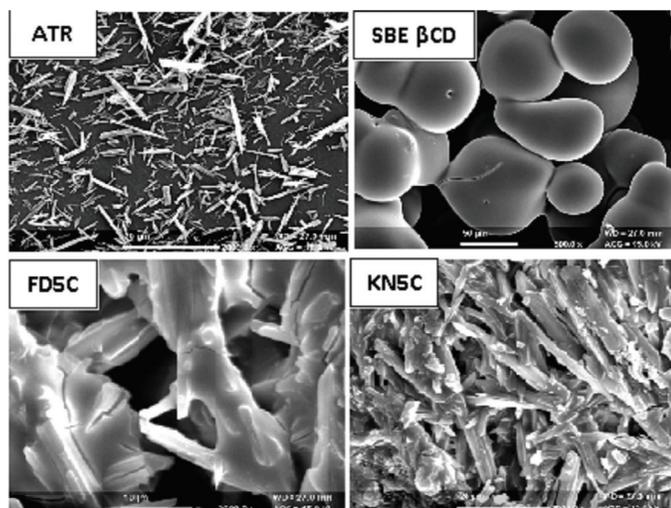


Figure 6. Scanning electron micrograph of ATR, SBE β -CD, FD5C, and KN5C.

to its melting point (Fig. 7). DSC thermogram of SBE β -CD showed (Fig. 8) sharp endothermic peak at 272.22 °C and broad endotherm at 96.03°C with enthalpy of fusion 49.733 J/g and 214.70 J/g, respectively, and subsequent to its melting point. DSC thermogram of FD5C showed two endothermic peaks (related to SBE β -CD) at 99.00°C and 240.06°C with enthalpy of fusion 113.56 J/g and 41.485 J/g (Fig. 9). A slight shift and very small peak of drug (at 163.03°C) in DSC thermogram of FD5C suggested strong interaction of drug with SBE β -CD. This is also supported by a shift in the endothermic peak of SBE β -CD at 272.22 °C to 240.06°C in FD5C which indicated formation of stable complex.

In vitro dissolution studies

In vitro drug release profile of pure ATR and various β -CD inclusion complexes and SBE β -CD inclusion complexes is shown in Figures 10 and 11, respectively. The lyophilized ATR- SBE β -CD inclusion complex (FD3C) exhibited 100% drug release in 2 hours while it is only 65% in pure drug. The drug release from lyophilized β -CD inclusion complex (FD5 β) showed 96% drug release in 2 hours. The kneading method is not supported as per the results of solubility studies and dissolution as well. The inclusion complexes formed by freeze drying/lyophilization exhibited significantly higher drug release in comparison to that formed by kneading method. 1:3 ratio of drug to cyclodextrins proved optimum (formation of stable inclusion complex), and it is also supported by dissolution results. The dissolution rate was increased with increase in concentration of β -CD (Figs. 10 and 11). The general solubility of chemically modified SBE β -CD is 70 g/100 ml where as in case of naturally occurring β -CD it is just 1.85 g/100 ml which clearly shows that SBE β -CD has more than 30 times greater solubility than β -CD; and the advantage of being amorphous in nature provides an opportunity to form better inclusion complex with higher dissolution rate (Devasari *et al.*, 2015; Lankalapalli *et al.*, 2018; Shiralashetti and Patil, 2014). On the basis of results of *in vitro* dissolution studies, the lyophilized batch FD3C will be utilized further for *in vivo* studies.

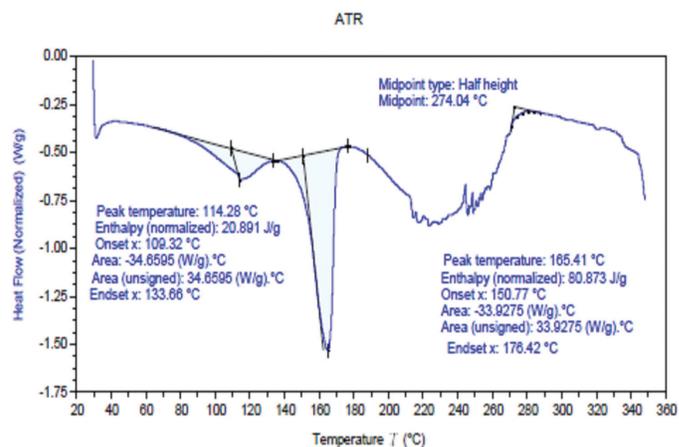


Figure 7.

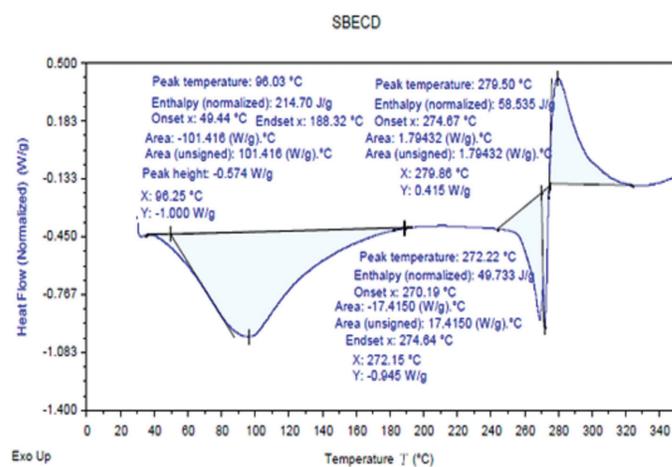


Figure 8. DSC thermogram of SBE β -CD.

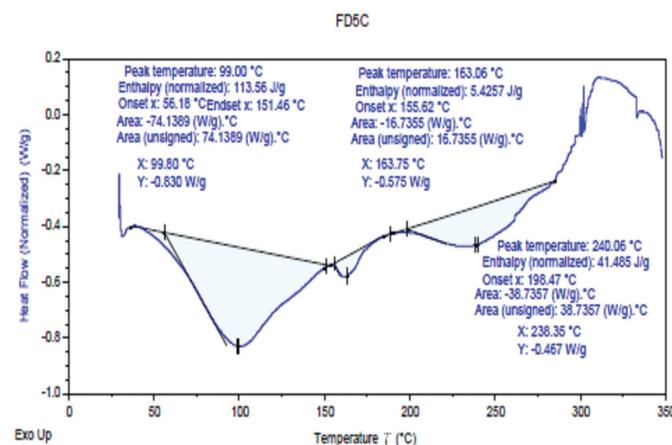


Figure 9. DSC thermogram of FD5C.

In vivo pharmacodynamics studies

Effect of optimized formulation of Atorvastatin on body weight gain and body mass index of rats

Obesity was induced in rats by feeding a high fat diet for 08 weeks. The mean body weights of the four experimental groups were same with minimum variability at the start of the study. A significant increase in body weight and BMI was observed in rats of HFD control group after 08 weeks, as compared to normal control group. Furthermore, the once daily per oral treatment with Atorvastatin (Pure drug, 10 mg/kg, *p.o.*) for 4 weeks significantly decreased the body weight and BMI as compared to HFD control group ($p < 0.01$). Also, the treatment of animals with optimized test formulation of Atorvastatin (FD3C) (10 mg/kg, *p.o.*) for four weeks shows significant ($p < 0.001$) difference in the decreased body weight and BMI, as compared to HFD control rats (Figs. 12 and 13).

Effect of optimized formulation of Atorvastatin on high fat diet induced changes in blood glucose level of animals

Random blood glucose levels were measured at the end of study (08 weeks). Feeding with high fat diet for 12 weeks significantly increased the blood glucose level in HFD control group as compared to normal control group. Furthermore, the once daily per oral treatment with Atorvastatin (Pure drug, 10 mg/kg, *p.o.*) for 4 weeks significantly decreased blood glucose level as compared to HFD control group ($p < 0.01$). Also, the treatment of animals with optimized test formulation of Atorvastatin (FD3C) (10 mg/kg, *p.o.*), for 4 weeks shows significant ($p < 0.001$) difference in blood glucose level, as compared to HFD control rats (Fig. 14).

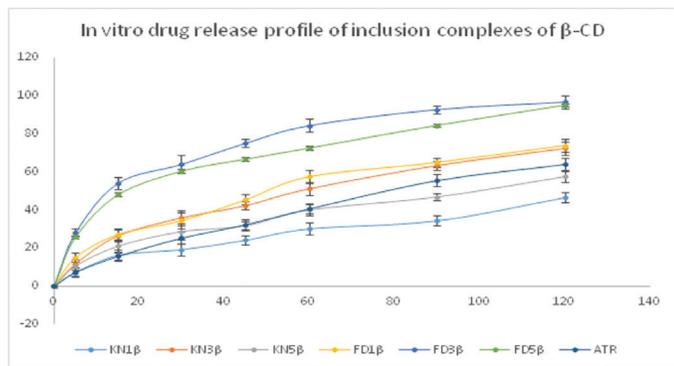


Figure 10. *In vitro* release profile of inclusion complexes of ATR and β -CD.

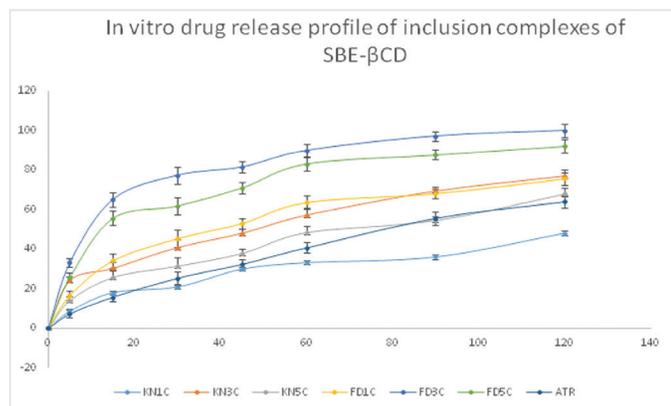


Figure 11. *In vitro* release profile of inclusion complexes of ATR and SBE β -CD

Effect of optimized formulation of Atorvastatin on high fat diet induced changes in Lipid Profile of animals

The evaluation of serum lipid profile of experimental animals was carried out for all groups. There was statistically

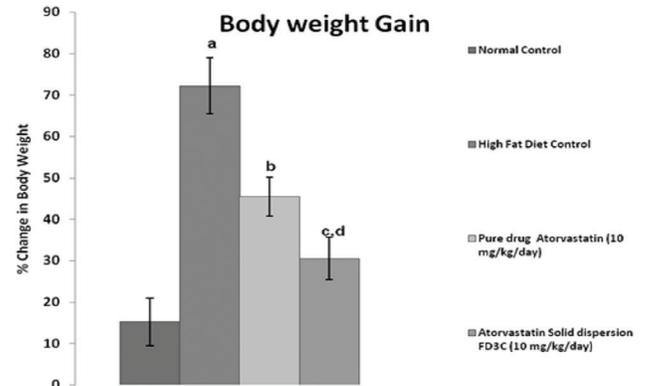


Figure 12. Effect of optimized formulation of ATR on % body weight gain of rats.

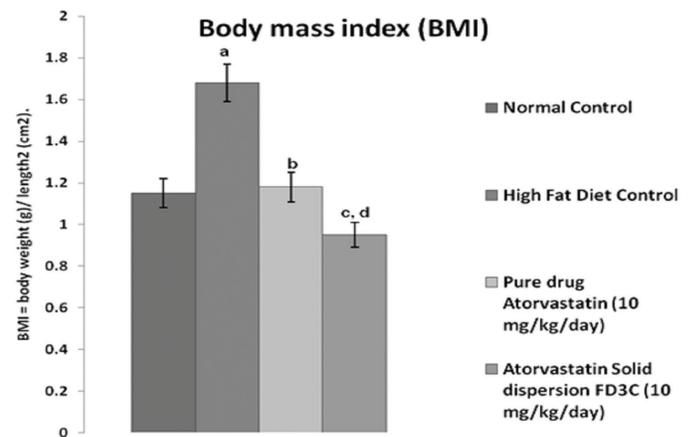


Figure 13. Effect of optimized formulation of Atorvastatin calcium on BMI of rats

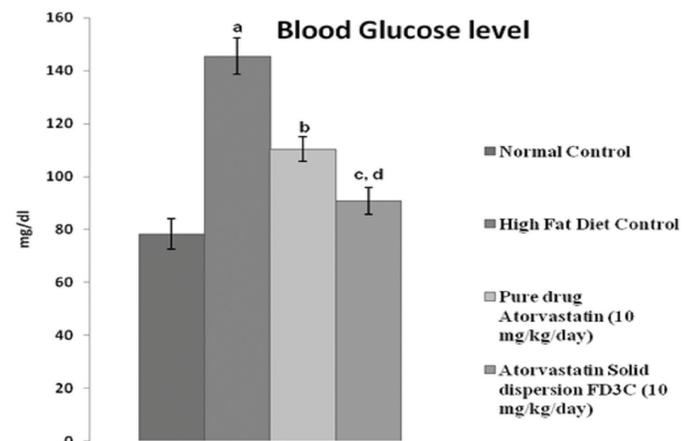


Figure 14. Effect of optimized formulation of Atorvastatin calcium on random blood glucose level of rats.

significant ($p < 0.01$) increase in TC, triglycerides (TG), Low density lipoprotein (LDL) along with decreased high density lipoprotein (HDL) in HFD control group, as compared to normal control group. Further, the once daily per oral treatment with Atorvastatin (Pure drug, 10 mg/kg, *p.o.*) for four weeks significantly decreased the levels of TC, TG, LDL with increase in HDL as compared to HFD control group ($p < 0.01$).

Also, the treatment of animals with optimized test formulation of Atorvastatin (FD3C) (10 mg/kg, *p.o.*), for 4 weeks significantly ($p < 0.001$) modulates the levels of TC, TG, LDL with increase in HDL as compared to HFD control rats (Table 2).

Effect of optimized formulation of Atorvastatin on histopathology

HFD consumption resulted in marked enlargement of adipocytes in highly fat diet control groups. More fat accumulation and consequently marked expansion of adipocytes size is seen in liver tissues. Distended adipocytes and extra fat deposits can be noticed in HFD groups when compared to normal control group. Administration of optimized test formulation of

Atorvastatin (FD5C) (10 mg/kg, *p.o.*) has considerably reduced the size of adipocytes, when compared to disease control group. The comparative microscopic representation of histologically examined livers is shown in Figure 15.

CONCLUSION

The modified form of β -CD proved as a prospective carrier system for poorly soluble Atorvastatin calcium. The freeze drying method for preparation of inclusion complexes proved better in terms of yield and dissolution as well. The modified form of β -CD, being amorphous and stable in comparison to pure β -CD, yielded better dissolution characteristics. The lyophilized inclusion complex FD3C (of ATR and SBE β -CD) lead to better hypolipidemic activity as seen in pharmacodynamic studies in comparison to ATR alone studies. Therefore CAPTISOL, i.e., SBE β -CD-based inclusion complexes can be potential carrier system for poorly soluble drugs and for also useful for oral drug delivery.

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CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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Table 2. Effect of various treatments on high fat diet induced changes in lipid profile of rats.

Parameter (mg/dl)	Groups			
	Control	High fat diet (HFD)	Pure drug (10 mg/kg/day)	Inclusion complex (10 mg/kg/day)
Total cholesterol	114.00 \pm 5.2	211.90 \pm 8.04*	147.6 \pm 7.25#	127.8 \pm 5.40 [!]
LDL cholesterol	40.52 \pm 1.74	115.62 \pm 3.8*	72.48 \pm 3.35#	47.40 \pm 2.24 [!]
HDL cholesterol	45.28 \pm 2.31	23.78 \pm 3.9*	37.5 \pm 2.8#	42.6 \pm 1.95 [!]
Triglyceride	82.21 \pm 3.5	233.53 \pm 7.51*	130.37 \pm 5.25#	97.20 \pm 3.82 [!]

*Indicates significant difference from the control group, at $p < 0.05$.

#Indicates significant difference from the HFD group, at $p < 0.01$.

! Indicates significant difference from the HFD group, at $p < 0.001$.

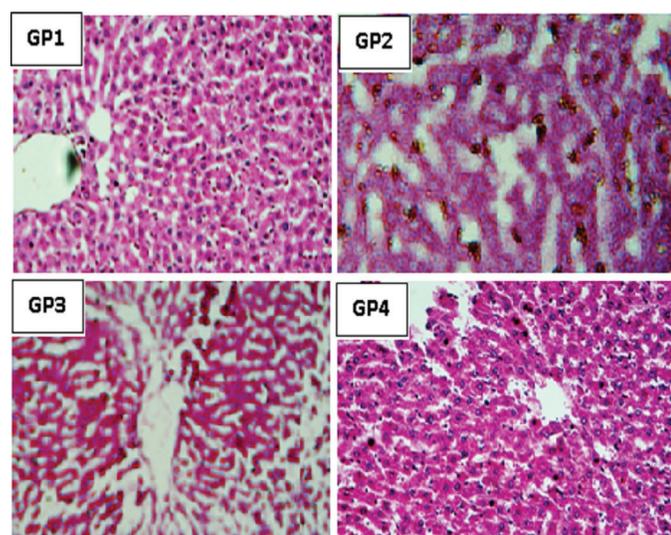


Figure 15. Gp 1- Normal hepatic adipocytes; Gp 2- Distended adipocytes showing extra fat deposits; Gp 3- Pure drug Atorvastatin treatment (comparatively large size of adipocytes); Gp 4- FD5C inclusion complex treatment (reduced size of adipocytes).

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