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Influence of atorvastatin calcium-cyclodextrin complexation on solubility, stability and pharmacodynamic activity

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Abstract

The objective of the investigation was to improve the solubility, stability and the dissolution rate, which would improve the bioavailability of atorvastatin calcium (ATN Ca), by complexation with hydroxypropyl β-cyclodextrin (HPβCD). Phase solubility profile indicated that the solubility of ATN Ca was significantly increased in the presence of HPβCD and was classified as AL-type. The complexes were prepared by physical mixing, kneading and spray drying methods and evaluated for solubility, intrinsic dissolution rate, fourier transform infrared spectroscopy, differential scanning calorimetry and powder X-ray diffractometry. In vitro studies showed that the solubility and dissolution rate of ATN Ca was significantly improved by complexation. Spray-dried product showed higher solubility and dissolution rate than other complexes. ATN Ca unit dosage form was developed and evaluated for physico-chemical properties, stability and dissolution rate. The stability of tablets was studied and no significant changes were detected in dissolution profile and drug content of tablets after 3 months. The in vivo studies of spray dried complex compared to ATN Ca in rats reveal that there was significant reduction ($p<0.05$) in cholesterol and triglyceride levels and significant improvement ($p<0.05$) in HDL level. ATN Ca- HPβCD complexes could be prepared with improved solubility, stability and hypolipidemic activity.

Key words: Atorvastatin calcium, hydroxypropyl β-Cyclodextrin, phase solubility, inclusion complex, spray drying.

Introduction

Atorvastatin calcium (ATN Ca) is [R-(R*, R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt, a selective, competitive HMG-CoA reductase inhibitor, a potent lipid-lowering agent and is used as hypolipidemic agent. It is also used in the treatment of osteoporosis, benign prostatic hyperplasia and Alzheimer's disease. ATN Ca rapidly absorbed after oral administration, but undergoes extensive first-pass metabolism in the gut wall and in the liver, that results an oral bioavailability of 14%. It is extensively metabolized by oxidation, lactonisation and glucuronidation, and the metabolites are eliminated by biliary secretion and direct secretion from blood to the intestine.

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ATN Ca is very slightly soluble in water, phosphate buffer (pH 7.4) and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol (Black et al.1998, Kerc et al. 2004).

Therefore, it is necessary to improve the solubility and dissolution rate of drug along with stability, which can lead to substantially enhancing its bioavailability. In recent years, cyclodextrin complexation has been successfully used to improve solubility, dissolution rate, chemical stability and bioavailability of a number of poorly soluble drugs.

β-cyclodextrins are β-1,4-linked cyclic oligosaccharides composed of seven D-glucopyranose units with a relatively hydrophobic central cavity and having the capacity to entrap poorly soluble drug molecules to form reversible noncovalent inclusion complexes. This may improve physical and chemical properties of the incorporated guest molecule allowing, for example the improvement of solubility, stability (Rajewski et al. 1996), in vivo drug delivery and bioavailability. However, it is known that the application of β-cyclodextrin in the pharmaceutical field is limited by its rather low aqueous solubility, which led to a search for more soluble derivatives of cyclodextrins (Szente et al. 1999, Martin et al. 2004, Masson et al. 1998 and Loftsson et al. 1996).

Among industrially produced, standardized, and available β-cyclodextrin (βCD) derivatives, the most important ones are the heterogeneous, amorphous, highly water-soluble 2-hydroxypropylated β-cyclodextrins (HPβCD). They are widely used in pharmaceutical field owing to its ability to stabilize the drug molecules. Due to their heterogeneity, these products cannot be crystallized, which is an important advantage. It is soluble in cold water as well as in organic solvent. It is available in more than 95 % isomeric purity for injectable drug formulation (Loftsson et al. 1996).

The objective of the study was to improve solubility and dissolution rate of poorly water soluble ATN Ca by complexation with HPβCD. The solid complexes were prepared by different methods; physical mixing, kneading and spray-drying at stoichiometric ratios. Phase solubility study, FTIR, DSC and X-ray diffraction studies confirmed the formation of such complex. In vivo study in rats was carried out for hypolipidemic activity. ATN Ca unit dosage form was developed and evaluated for physicochemical properties, stability and dissolution rate.

Material and Methods

Material

Atorvastatin Calcium was generously provided by Biocon Pharmaceutical Ltd., Bangalore, India. Hydroxypropyl β-Cyclodextrin (HPβCD) was a gift sample by M/s Roquette Ltd. (France). Dulbecco's buffer (pH 6.8 and 7.4) was purchased from Himedia, Mumbai, India. All other chemicals and reagents used were of analytical grade.

Phase Solubility Studies

Phase solubility studies were carried out according to the method reported by Higuchi and Connors (Higuchi et al. 1965). Excess amount of the ATN Ca was added to aqueous solutions containing increasing concentrations of HPβCD (0.5 mM to 3 mM). The HPβCD solutions were prepared in distilled water, pH 1.2 and phosphate buffer (pH 7.4). The flasks were sealed and shaken for one week to ensure equilibrium. The samples were centrifuged at 4000 rpm and filtered through 0.45 μm membrane filter, appropriately diluted and analyzed spectrophotometrically at 246 nm. The apparent stability

constant (Ks) (Table 1) of the complexes was calculated from the slope of the phase solubility diagram (Higuchi et al. 1965, Ann et al. 2004).

$$K_{1:1} = \text{slope} / S0 (1 - \text{slope})$$

Table 1. Composition of ATN Ca single tablet.

S.No.	INGREDIENTS	AMOUNT (mg)
1.	ATN Ca (or) Drug-HPβCD complex equivalent to ATN Ca	10
2.	Lactose Monohydrate	20
3.	Microcrystalline Cellulose	20
4.	Calcium carbonate	16
5.	Crosscarmellose sodium	1.4
6.	PVP K-30	2
7.	Magnesium stearate	0.6

Preparation of inclusion complexes

All the binary mixtures were prepared in the 1:1 molar ratio between drug and HPβCD on the basis of the results obtained from the preliminary phase solubility studies.

Physical mixture

Physical mixture (1:1) was prepared by simple mixing ATN Ca and HPβCD using mortar and pestle for 10 min, the powders of both components previously sieved through a 250 μm sieve (Zingone et al. 2005).

Kneaded complex

HPβCD and distilled water were mixed together in a mortar so as to obtain homogeneous paste. ATN Ca was slowly added and the mixture was then ground for 15 min. During this process, an appropriate quantity of water was added to the mixture in order to maintain a suitable consistency. The resulting paste was dried in an oven at 45° C for 48 h and the dried complex was pulverized into a fine powder and sieved through a 250 μm sieve (Stephane et al. 2005).

Spray-dried complex

Spray-dried product (1:1) was prepared by dissolving weighed quantity of atorvastatin calcium and HPβCD in 100 ml methanol. The resulting solution was stirred and subsequently spray-dried using laboratory scale spray dryer (Jay Instruments and system Pvt. Ltd, Mumbai, India), under the following conditions: flow rate of the solution 5ml/min; inlet temperature 78 ^{0}C; outlet temperature 50 ^{0}C; aspirator-120mmWC (Francisco et al. 2001).

Characterization of ATN Ca - HPβCD complexes

Drug content of complex

Samples of each binary mixture were assayed by dissolving weighed amounts (20 mg) in 10 ml of methanol. The solution was filtered, diluted and the drug content was determined spectrophotometrically at 246 nm.

Saturation solubility of pure drug and drug complex

The saturation solubility of ATN Ca and different complexes were determined by equilibrating excess powder in water and different buffer solutions (pH 1.2, 4.5 and 7.4) for 48 h on a mechanical shaker at 37° C. The samples were centrifuged at 4000 rpm for 10 min; supernatant was filtered through 0.45 μm membrane filter (Zingone et al. 2005). The filtrate was collected and assayed for ATN Ca spectrophotometrically at 246 nm.

Intrinsic dissolution rate (IDR)

In vitro IDR was measured using USP 30 dissolution apparatus type II. Pure drug and different batches of complexes were compressed in 13 mm IDR cell by using KBr press at 100 kg/cm^2 for one min and was placed in 900 ml phosphate buffer pH 7.4. The dissolution medium was equilibrated at 37 ± 0.2° C and stirred at a speed of 100 rpm. Aliquots were collected periodically and replenished with fresh dissolution media. Drug concentration was determined spectrophotometrically at 246 nm. Data analysis was carried out using PCP-Disso software (V3, Poona College of Pharmacy, Pune, India).

Fourier transformed infra red spectroscopy (FTIR)

All the binary mixtures were prepared by different methods were analyzed by FTIR. The binary mixtures along with KBr were subjected to a pressure of 150 kg/cm^2 in a KBr press (Spectra Lab. India.). The pellets were then analyzed by using FTIR (V 5300, JASCO, Japan) and the range from 4000 to 400 cm^{-1} was selected (Seoung et al. 2007).

Differential scanning calorimetry (DSC)

Thermal characteristics of drug and drug cyclodextrin complex were studied using differential scanning calorimeter equipped with an intracooler (METLLER Toledo DSC 821^e module controlled by STAR^e software, Toledo GmbH, Switzerland). Indium/Zinc standards were used to calibrate the DSC temperature and enthalpy scale. The samples were hermetically sealed in aluminum pans and heated at a constant rate of 5^0 C/min over a temperature range of 25 to 250 ^{0}C. Inert atmosphere was maintained by purging nitrogen gas at flow rate of 20 ml/min. An empty aluminum pan was used as reference (Longxiao et al. 2006, Gladys et al. 2003).

Powder X-ray diffractometry (PXRD)

The X-RD patterns were reported on X-ray Diffractometer (PW 1729, Philips, Netherland) the samples were irradiated with monchromatized CuKα radiation (1.542 A°) and analyzed between 2-80° 2θ. The voltage and current used were 30 kV and 30mA respectively. The range and the chart speed were 5 x 10^3 CPS and 10 mm/ °2θ, respectively (Nalluri et al. 2003).

Preparation of tablets

Tablets were prepared by wet granulation technique and composition of a single tablet was shown in Table 1. The tablets were evaluated for weight and thickness variation, friability, hardness, content uniformity and disintegration time.

In vitro dissolution studies

The dissolution studies were performed using USP 30 type II dissolution test apparatus (TDT- 06P, Electrolab, India). The tablets of different formulations equivalent to 10 mg atorvastatin calcium was placed in the dissolution vessel containing 900 ml phosphate buffer (pH 6.8) maintained at 37^{0}C±0.5^{0}C and stirred at 50 rpm. Samples were collected periodically and replenished with a fresh dissolution medium. Concentration of drug was determined spectrophotometrically at 246 nm. Percent release of drug was calculated by using PCP-Disso software (V3, Poona College of Pharmacy, Pune, India.).

Stability studies

The stability studies were carried out according to ICH guidelines. The samples were stored at 40 ± 0.5° C/75 ± 5 % R.H for 90 days. Samples with drawn after one month and three months were evaluated for disintegration time, drug content and dissolution rate.

In vivo studies

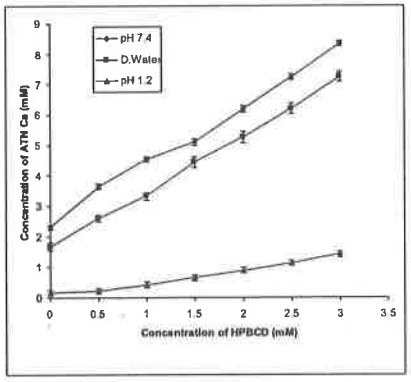
The hypolipidemic activity of inclusion complexes was determined in comparison with pure atorvastatin calcium in healthy albino rats (Wistar strain) of either sex, weighing between 190 and 250 g. The animals were procured from National Toxicology Center (Pune, India). General and environmental conditions were strictly monitored. The institutional animal ethics committee of Poona College of Pharmacy, Pune, India, approved the research protocol of the animal experimentation. The animals were divided into three groups and each group was having six animals. The animals were starved for 18 hours and then injected 200 mg/kg Triton WR 1339 (iso octyl-polyoxyethylene phenol) intraperitonealy. Serum cholesterol levels increased sharply 2-3 times after 24h. Reference and test groups additionally received aqueous suspensions of pure drug and spray dried complex (equivalent to 10mg/kg body weight) respectively, prepared by using 1 % w/v gum acacia as a suspending agent. Blood samples were collected under light ether anesthesia by retro orbital puncture at 0, 6, 24 and 48 h. The serum samples were analyzed for total cholesterol, triglycerides (TG) and high density lipoprotein (HDL) levels by the in vitro diagnostic kit (ACCUREX BIOMEDICAL PVT.LTD., Mumbai, India). The statistical analysis for the determination of differences in lipid profiles of treatment and control groups was done by one way ANOVA followed by Dunnet's test and $p< 0.05$ was taken as significant (Anshuman et al. 2005, Vogel et al. 1997).

Results and Discussion

Phase solubility studies

The phase solubility diagrams of ATN Ca: HPβCD was obtained by plotting the changes in guest solubility as a function of HPβCD concentration. Figure 1 illustrated that the apparent solubility of ATN Ca increases linearly as a function of HPβCD over the entire concentration range and was characteristic of AL type of curve that suggested formation of a water-soluble complex. The slope values obtained were less than 1, which indicated that inclusion complex in the molar ratio of 1:1 between the guest and the host molecule was obtained irrespective of the pH.

Figure 1. Phase solubility studies of ATN Ca with HPβCD.



Stability constant

The extent of complexation in aqueous media (i.e., the stability of the formed complex) was characterized by the stability constant Ks. The Ks determination was based on the solubility diagrams, which required calculations involving drug solubility. Hence, Ks values were calculated according to the equation of Higuchi and Connors from the initial straight-line portion of the solubility diagrams by assuming that a 1:1 complex was initially formed (the

slope was less than 1). The apparent stability constants Ks of the 1:1 complexes at each pH value were calculated from the slopes of the solubility diagrams. The Ks values decreased with increase in the pH (Table 2).

Table 2. ATN Ca saturation solubility, slope, *K*s and correlation coefficient (R^2) from phase solubility diagrams.

Buffer	Solubility of ATN Ca (Mean ± SD)	Slope	Ks (M^{-1})	R^2
pH7.4	0.90 ± 0.09	0.192	102	0.999
Distilled Water	0.63 ± 0.09	0.169	122.4	0.998
pH 1.2	0.09 ± 0.01	0.042	265.2	0.996

This phenomenon could be due to the ionization of the acidic atorvastatin at pH > pka. Ionic form of the drug showed lower hydrophobicity and weaker interactions with the hydrophobic cavity of HPβCD than the unionized drug. The results reveal that the pH played an important role in determining the strength of complexation between drug and HPβCD.

Drug content of complex

The drug content of physical mixture, kneaded and spray-dried complexes were found to be 99.0 %, 95.2 % and 96.9 % respectively and were in good agreement with the theoretical and actual drug content.

Saturation solubility of complex

The saturation solubility of pure drug, physical mixture, kneaded complex and spray-dried complex were obtained over a range of pH 1.2 to 7.4 as shown in Table 3.

Table 3. Saturation solubility (mg/ml) of atorvastatin calcium complexes with HPβCD.

SOLVENTS	SD Complex (S±SD)	Kneaded product (S±SD)	Physical mixture (S±SD)	Pure Drug (S±SD)
Phosphate buffer(pH 7.4)	28.76±0.5	16.66±0.59	3.83±0.09	0.90±0.1
Distilled Water	24.78±0.9	14.46±0.67	2.16±0.05	0.63±0.1
Acetate buffer (pH 4.5)	16.14±0.2	10.12±0.12	1.98±0.02	0.31±0.2
0.1 N HCl (pH 1.2)	3.86±0.16	2.83±0.10	0.40±0.01	0.09±0.01

The saturation solubility of atorvastatin calcium increases from 0.09 ± 0.01 mg/ml to 0.90 ± 0.1 mg/ml (10- fold). This pH solubility profile is consistent with the ionization of the carboxyl group. This study supports interconversion kinetics, equilibrium, and solubility of the lactone and hydroxy forms of the atorvastatin sodium (Kearny et al. 1993). It can be seen that an increase of the solubility values was obtained from all the binary mixtures. This was probably due to the presence of hydrophilic cyclodextrin and a better wettability of the drug. Spray-dried product showed a 39-fold and 32- fold increase in water and phosphate buffer (pH 7.4), respectively than pure drug, attributable to the formation of inclusion complex.

Intrinsic dissolution profile

The intrinsic dissolution studies of pure drug, physical mixture, kneaded product and spray dried product were performed in phosphate buffer pH 7.4. Pure ATN Ca showed a flux of 102 $\mu g\ cm^{-2}\ h^{-1}$ as shown in Figure 2. The enhancement in intrinsic dissolution rate was dependent

on the preparation method. The physical mixture and kneaded product showed a slight increase in the dissolution rate. This result was due to the solubilizing effect of the HPβCD and also by improved wettability of the drug. A significantly higher intrinsic dissolution rate was observed in spray dried product (258 μg cm^{-2} h^{-1}) due to the formation of soluble inclusion complex, amorphisation of the drug and consequently higher solubility and better wettability.

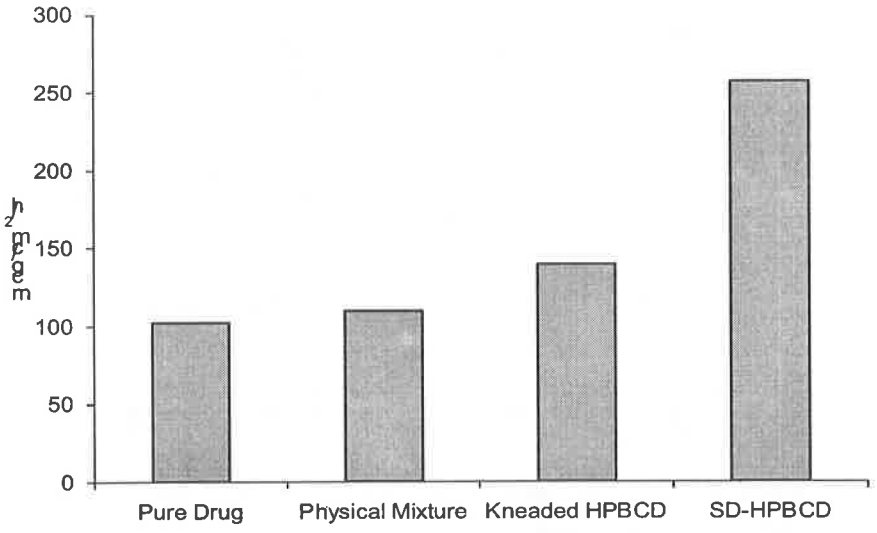


Figure 2. Intrinsic dissolution rate in pH 7.4 buffer.

FTIR study

ATN Ca showed characteristic bands at 3063, 1658, 1593, 1226, 1159 and 841 cm^{-1}. The IR spectra of HPβCD showed prominent absorption bands at 3414, 2933, 1164, 1083 cm^{-1}. Physical mixture showed bands at 3387, 1658, 1585, 1313, 1157 and 1028 cm^{-1}. There was slight shift in the absorption spectrum of the drug and it indicates that there was no strong interaction between the drug and the cyclodextrin molecule (Figure 3).

The spectra of kneaded complex also showed the same bands. The IR spectra of spray-dried product revealed bands at 3364, 1651 and 1577 cm^{-1}. These were attributed to interaction between the drug and OH groups of the cyclodextrin. Absence of bands at 3063, 1313 and 841cm^{-1} indicated that the vibrating and bending of the guest molecule (ATN Ca) was restricted due to the formation of an inclusion complex and the aromatic rings of atorvastatin being inserted into the cavity of the cyclodextrins (Narender Reddy et al. 2004, Sanjula et al. 2005).

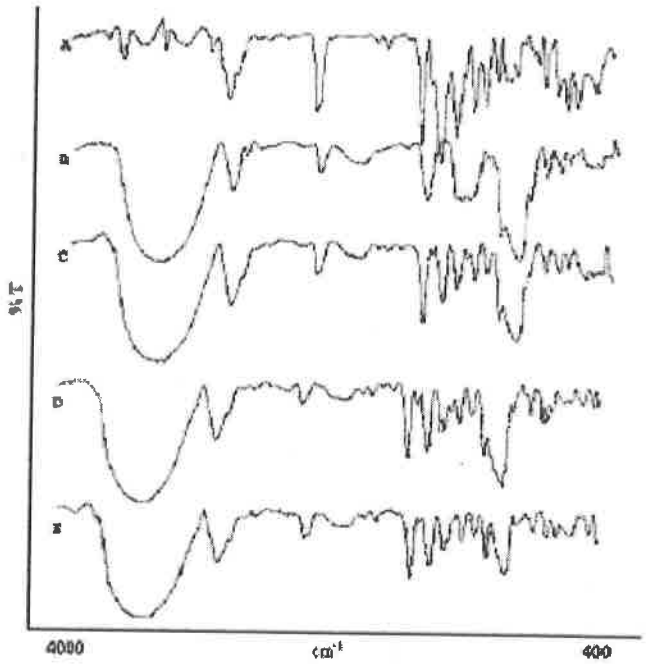
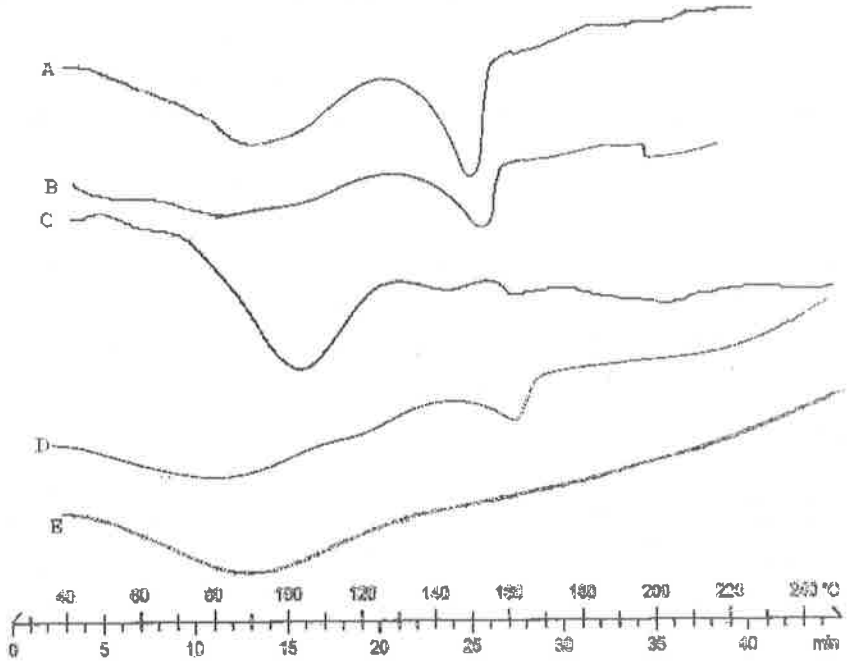


Figure 3. FTIR graphs of ATN Ca and HPβCD complexes: A) Pure Drug B) HPβCD C) Physical Mixture D) Kneaded form E) Spray-dried.

DSC studies

The DSC thermograms of pure drug, physical mixture, HPβCD, kneaded product and spray-dried complex were shown in Figure 4. Pure drug characterized by a single, sharp melting endotherm at 158.57^0C (ΔH –47.42 Jg^{-1}). The DSC curve of HPβCD exhibited a very broad endothermal phenomenon between 60° C and 120° C due to loss of water. A broad endotherm was observed in the thermograms of physical mixture, kneaded and spray dried complex due to loss of water during the heating cycle. Drug melting peak was observed in case of physical mixture and kneaded product. Thus only weak interactions can be postulated in the case of physical mixture and kneaded product. However no endotherm characterizing the melting transition was noted in the thermogram of spray-dried complex suggesting that the complete inclusion complex without free ATN Ca was formed. This is suggestive of the formation of an amorphous inclusion complex with the molecular encapsulation of the drug inside the HPβCD cavity. The previously published study on the DSC thermogram of nimesulide and cyclodextrin complex, have reported that the characteristic peak of nimesulide completely disappears and concluded that a true inclusion complex was formed (Narender Reddy et al. 2004).

Figure 4. DSC studies of ATN Ca and HPβCD Complexes: A) Pure Drug B) Physical mixture C) HPβCD D) Kneaded product E) Spray-dried product.



PXRD studies

A supporting evidence for the formation of an amorphous inclusion complex between drug and HPβCD was obtained from powder X-ray diffraction pattern. The powder X-ray diffraction patterns of pure drug, physical mixture, kneaded product and spray-dried complex are shown in Figure 5.

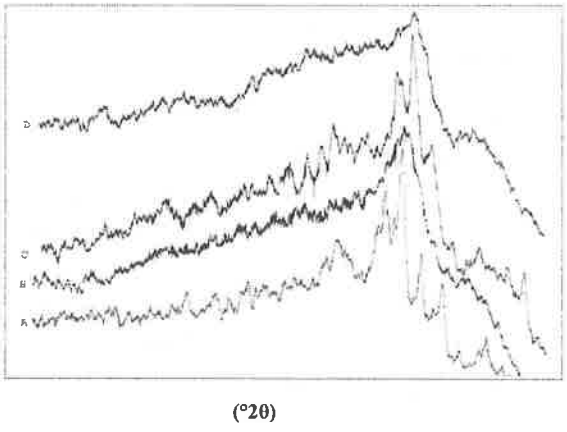


Figure 5. PXRD studies of ATN Ca and HPβCD complexes: A) Pure Drug B) HPβCD C) Kneaded product D) Spray dried product.

The PXRD pattern of pure drug presented several diffraction peaks indicating the crystalline nature of the drug. The drug shows 2θ values at 9.15, 9.47, 10.26, 11.85, 12.19, 17.07, 19.48, 21.62, 22.96, 24.43 28.92 and 29.23. HPβCD presented an amorphous X-ray diffraction

pattern. The PXRD patterned of kneaded product showed some of characteristic peaks, indicating the presence of ATN Ca in crystalline state. In contrast, spray-dried complex showed complete absence of sharp peak and some peaks with reduced intensity at 17.075, 19.48, 21.62, and 22.96, suggesting that the existence of amorphous state of drug. This reduction in crystallinity can be attributed to the spray-drying treatment. This phenomenon confirmed that atorvastatin calcium - HPβCD forms an efficient inclusion complex in the solid state (Sanjula et al. 2005).

Physical properties of tablets

The tablets prepared by wet granulation method using pure drug, kneaded product and spray-dried complex were analyzed for the physical properties as per USP 30. Weight variation, thickness variation, hardness, friability, disintegration time and drug content were shown in Table 4.

Table 4. Physical properties of tablets.

Batch No.	Weight (mg) Mean±SD	Thickness (mm) Mean±SD	Hardness (Kp) Mean±SD	Friability (%)	D.T. (Sec)	Drug Content (%)
B1	69.87±2.69	2.47±0.94	4.50±1.28	0.328	62	107.1±2.4
B2	68.87±2.47	2.72±0.96	4.60±1.18	0.301	65	101.5±1.5
B3	68.25±2.53	2.36±1.01	4.70±1.13	0.284	60	92.85±1.8
B4	69.37±1.72	2.51±0.86	4.50±0.84	0.265	61	96.4±2.2
B5	67.84±2.65	2.46±1.02	5.10±0.54	0.230	54	98.6±1.4
B6	68.32±1.85	2.58±0.68	4.90±0.68	0.195	56	102.5±2.6

B1: Pure drug, B2: Pure drug with buffering agent, B3: Kneaded product, B4: Kneaded product with buffering agent, B5: Spray-dried product, B6: Spray-dried product with buffering agent.

It showed good uniformity of weight and thickness of all the formulations. The tablets also exhibited good mechanical properties with regard to both friability and hardness. Tablets containing spray dried complex showed good disintegration time than the tablets containing pure drug. This could be attributed to that better wettability due to presence of cyclodextrin.

In vitro dissolution studies of tablets

In vitro dissolution study was carried out in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8) for the tablets prepared from kneaded, spray dried complex and further compared with dissolution of marketed product (Figure 6 a-b.). The percentage drug release of B3 and B5 were found to be 68.3, 82.4; 94.2, 100.8 % in 0.1 N HCl and phosphate buffer (pH 6.8) respectively. The results reveal that the dissolution rate was faster in phosphate buffer (pH6.8) as compare to acidic media; it might be due to acidic nature of drug (weak acid, pKa 4.46). In phosphate and acidic media, dissolution of B5 showed dramatic enhancement as compared to B3 and marketed product. This could be due to formation of inclusion complexation with HPβCD (Aly et al. 2003).

Stability studies

The disintegration time, in *vitro* release and percentage drug content results (Table 5) reveal that after one month and three months there was no significant ($p>0.05$) difference in disintegration time , *in vitro* release and percentage drug content. Thus the complexation of ATN Ca with HPβCD was found to be stable.

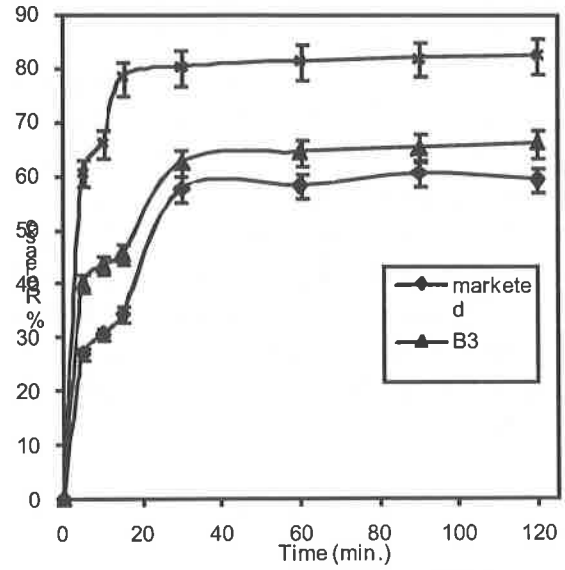


Figure 6a. Dissolution profiles of tablets prepared using kneaded complex (B3), spray dried complex (B5) and marketed formulation in 0.1N HCl (pH 1.2).

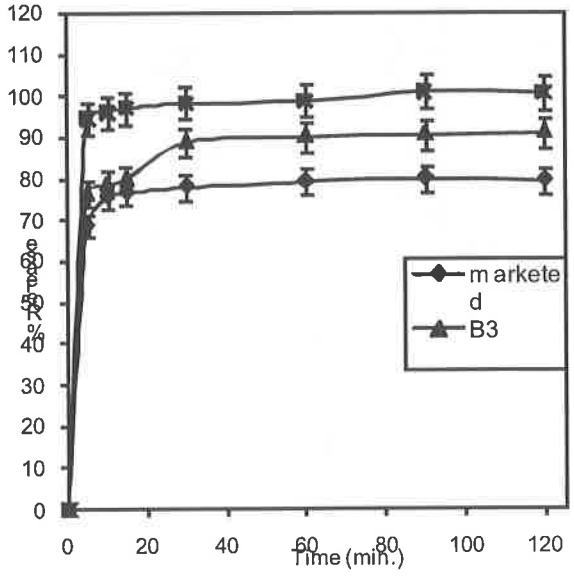


Figure 6b. Dissolution profiles of tablets prepared using kneaded complex (B3) spray dried complex (B5) and marketed formulation in phosphate buffer (pH 6.8).

In vivo studies

A hypolipidemic drug like ATN Ca was known to reduce elevated total cholesterol and triglycerides (TG) levels in blood. At the same time they cause elevation of HDL levels, which promote the removal of cholesterol from peripheral cells and facilitate its delivery back to the liver (Vogel and Vogel 1997). This pharmacodynamic effect is reported to be dose dependent

hence, was used as a basis for the comparison of in vivo performance of pure ATN Ca and spray-dried complexes with HPβCD. The serum lipid profiles of all the experimental groups at different time intervals are presented in Table 6. The results showed that there was significant decrease ($P<0.05$) in serum cholesterol and TG levels and increase ($P<0.05$) in HDL levels (Fig 7 a-c). Triton WR-1339 treated group (AP2) showed significant increase ($P<0.05$) in the cholesterol and TG levels as compared to reference group (AP3) and test group (AP4). The results suggest that spray dried complex showed greater bioavailability as the complex is having more solubility and dissolution rate. Thus the serum profiles of cholesterol and triglycerides were reduced significantly and the HDL was increased significantly. Therefore it can be concluded that the spray dried complex is having promising role in enhancing the solubility and thus subsequent improvement in the bioavailability of ATN Ca which could efficiently control the serum lipids.

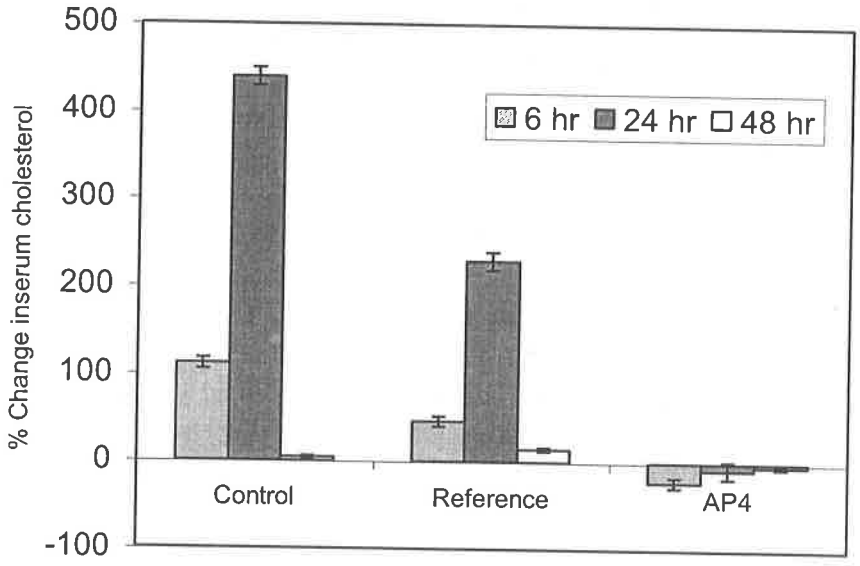


Figure 7a. Percent change in serum cholesterol levels of experimental groups at different time intervals.

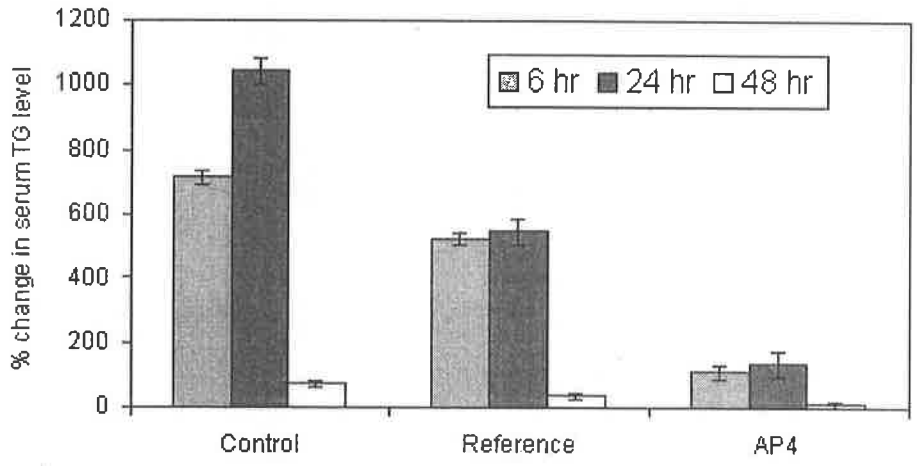


Figure 7b. Percent change in serum TG levels of experimental groups at different time intervals.

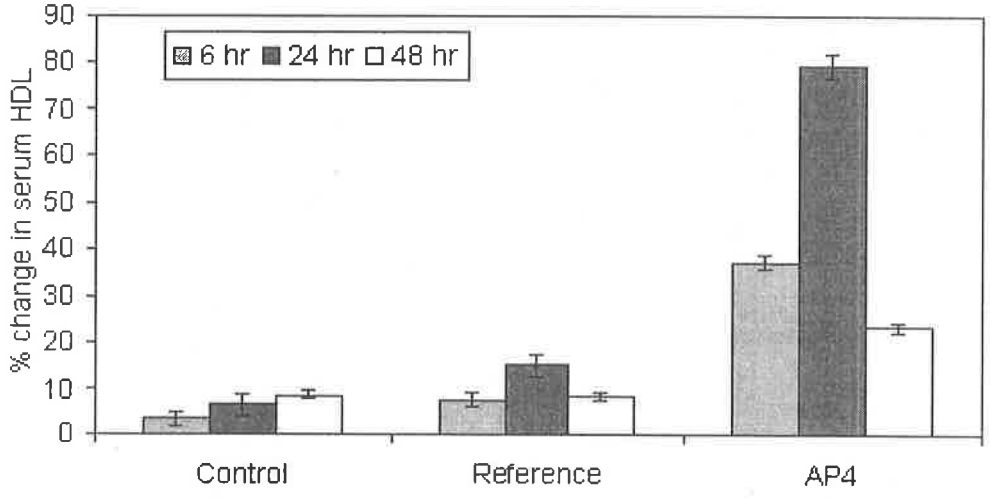


Figure 7c. Percent change in serum HDL levels of experimental groups at different time intervals.

Table 5. Characterization of different formulation during stability study at differant time interval.

Batch No.	Dissolution at the end of 60 min.			Disintegration Time (sec.)			Drug Content (%)		
	Day 0	Day 30	Day 90	Day 0	Day 30	Day 90	Day 0	Day 30	Day 90
B1	78.4±1.2	76.1±1.0	75.5±1.4	62	64	65	107.1±2.4	105.4±2.9	105.2±2.7
B2	68.2±0.9	68.0±0.6	67.8±0.7	65	66	64	101.5±1.5	102.0±2.2	101±2.1
B3	92.6±0.8	92.9±1.0	91.6±1.2	60	62	61	92.85±1.8	92.0±2.7	91.5±3.2
B4	88.0±0.8	86.5±1.3	84.7±1.3	61	60	63	96.4±2.2	95.6±1.9	94.5±2.4
B5	99.7±1.6	99.0±0.7	98.2±0.9	54	53	55	98.6±1.4	98.5±2.5	98.2±1.6
B6	93.4±1.2	92.1±1.5	90.3±1.5	56	56	58	102.5±2.6	101.3±2.1	101.3±1.2

Table 6. Serum cholesterol, triglyceride and HDL profiles of various experimental groups at different time intervals.

Group No.	Serum Cholesterol (h)				Serum Triglyceride(h)				Serum HDL(h)			
	0	6	24	48	0	6	24	48	0	6	24	48
AP2	44.3±1.8	93.8±3.9	239.2±10.2	46.2±1.5	62.7±7.4	511.3±18.9	717.5±23.5	108.9±7.5	26.8±1.7	27.6±1.1	28.4±1.1	29.1±2.1
AP3	40.8±3.2	59.6±4.1	134.0±8.3	46.7±2.5	63.5±8.1	398.5±12.4	413.2±18.2	85.4±6.7	30.1±1.8	32.3±1.2	34.7±1.9	32.6±1.7
AP4	50.1±1.9	38.7±2.6	46.2±3.8	48.2±2.2	77.4±6.8	161.7±10.1	181.4±12.5	85.2±6.2	32.5±1.4	44.6±2.1	58.2±1.9	40.1±1.7

Conclusions

The inclusion complexes of ATN Ca with HPβCD could be prepared by the spray-drying method in a molar ratio of 1:1. The inclusion complexes were found to have improved in vitro drug release compared with the pure drug. The results clearly demonstrated a significant decrease in the hypolipidemic activity in rats. Further studies are recommended to prove their therapeutic utility in human beings.

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