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# PREPARATION AND SOLID STATE CHARACTERIZATION OF ATORVASTATIN NANOSUSPENSIONS FOR ENHANCED SOLUBILITY AND DISSOLUTION

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**ABSTRACT:** In this study, an attempt was made to improve the solubility and dissolution characteristics of a poorly soluble drug (atorvastatin calcium) using nanosuspension technology. Nanoparticles were characterized in terms of size and morphological characteristics. Saturation solubility and dissolution characteristics were investigated and compared to the commercial drug. Crystallinity of the drug was also evaluated by performing thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) to denote eventual transformation to amorphous state during the homogenization process. Through this study, it has been shown that the crystalline state of the drug is reduced following particle size reduction and the dissolution rates of amorphous atorvastatin calcium nanoparticles were highly increased in comparison with commercial drug by the enhancement of intrinsic dissolution rate and the reduction of particle size, resulting in an increased specific surface area
*Keywords:* Nanoparticles; Drugs; High pressure homogenization; Dissolution; Crystallinity; Atorvastatin

## INTRODUCTION

One of the main problems responsible for the low turnout in the development of new molecular entities as drug formulations is poor solubility and poor permeability of the lead compounds. The increasing frequency of poorly water soluble new chemical entities exhibiting therapeutic activity is of major concern to the pharmaceutical industry. Various formulation parameters that play a crucial role for successful formulation are aqueous solubility, stability at ambient temperature and humidity, photo stability, compatibility with solvents and excipients etc. Of these, solubility is the most important property for developing formulations. A major hurdle that has prevented the commercialization of many promising poorly soluble drugs is dissolution rate limited bioavailability.. Many approaches have been developed to improve solubility and to enhance the dissolution rate and oral bioavailability of poorly soluble drugs e.g. salt formation[1,2], solid dispersion[3,4], inclusion complex[5,6], microemulsion[7,8], micronization[9,10], etc. Physical modifications often aim to increase the surface area, solubility and wettability of the powder particles and are therefore focused on particle size reduction or generation of amorphous states[11] In many studies, it is reported that amorphous systems is efficient for the enhancement of dissolution and bioavailability[2,13]. In addition, it was reported[14] that 10–1600 folds of solubility enhancement could be achieved by the use of amorphous systems.

Atorvastatin, as a synthetic lipid-lowering agent, is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMGCoA) reductase which catalyzes the conversion of HMG-Co A to mevalonate, an early rate-limiting step in cholesterol biosynthesis[15]. Atorvastatin 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. The intestinal permeability of atorvastatin is high

at the physiologically relevant intestinal pH[16]. However, it is reported that the absolute bioavailability (*F*) of atorvastatin is 12% after a 40mg oral dose[17]. In the present study, an attempt was made to improve the dissolution of atorvastatin calcium using high pressure homogenization technique.

## MATERIALS AND METHODS

Atorvastatin calcium (AC) was obtained as gift sample from M/s.Caplin point, Pondicherry, India. Polaxamer 188 was a gift sample from M/s.Colorcon, Goa, India. All other chemicals and solvents used are of analytical grade.

### *Preparation of drug nanosuspensions[18]*

Atorvastatin powder (5%w/v) was dispersed in an aqueous surfactant solution (0.5% w/v, suspensions) under magnetic stirring (500 rpm). After dispersion, a first size-reduction step using an Ultra-Turrax T25 Basic homogenizer (IKA-Werke, Staufen, Germany) at 24,000 rpm was conducted on the suspension (in an ice bath to prevent sample temperature increase). The obtained pre-mix was homogenized at room temperature using a Micron LAB 40 (APV Systems, Unna, Germany). At first, 2 cycles at 150 bar and 2 cycles at 500 bar as premilling steps were applied, then 10 cycles at 1500 bar were run to obtain the nanosuspension. Samples were withdrawn at each size reduction steps for size distribution analysis. The nanoparticles were obtained in a dry form by spray-drying using a Büchi B191a Mini Spray- Dryer (Büchi, Flawil, Switzerland). Suspensions were passed at a spray rate of 3.5 ml/min. The drying temperature was set at $115^0$.C. Spray airflow was set at 800 l/h and drying airflow was set at $35m^3$/h.

### *Particle size and shape characterization*

The particle size analysis was performed laser diffractometry (LD) using the Mastersizer E (Malvern Instruments). The LD yields a volume distribution.The particle size, d(*v*; 0.1) (size of the particles for which 10% of the sample volume contains particles smaller than d(v; 0.1), d(*v*; 0.5) (size of the particles for which 50% of the sample volume contains particles smaller than d(v; 0.5), and d(*v*; 0.9) (size of the particles for which 90% of the sample volume contains particles smaller than d(*v*; 0.9)) were used as characterization parameters. Morphological evaluation of nanoparticles was conducted through scanning electron microscopy (SEM) (Hitachi S-4200) following platinum coating.

### *Dissolution Studies[18]*

An USP dissolution apparatus (Electrolab, India) Type II (paddle method) at rotation speed of 50 rpm was used for in vitro testing of drug dissolution from the various formulations obtained after each size reduction step. Dissolution was carried out on an equivalent of 10 mg of atorvastatin (in powder state). Deionised water was used as the dissolution medium. The volume and temperature of the dissolution medium were 900 ml, and $37.0\pm0.2^0$C, respectively. Samples were withdrawn at fixed times and were filtered and assayed through ultraviolet absorbance determination at 245 nm using a Shimadzu UV/vis Spectrophotometer. The mean results of triplicate measurements and the standard deviation were reported.

### *Saturation Solubility studies*

Saturation solubility measurements were assayed through ultraviolet absorbance determination at 245 nm using a Shimadzu UV/vis Spectrophotometer. The dry powder (after water removal) obtained after each size reduction step and the pure drug saturation solubility study was performed as reported by J.Hecq et.al[19]. Weighed amount of AC (pure drug) and nanoparticles equivalent to 20 mg of the drug were separately introduced into 25- ml stoppered conical flasks containing 10 ml of distilled water. The sealed flasks were agitated on a rotary shaker for 24 h at 27° C and equilibrated for 2 days. An aliquot was passed through 0.1µm membrane filter (Millipore Corporation) and the filtrate was suitably diluted and analyzed on a UV Spectrophotometer at 245 nm. The mean results of triplicate measurements and the standard deviation were reported.

### *Characterization of Nanoparticles*

### *Differential scanning calorimetry (DSC)[20]*

Thermal properties of the powder samples were investigated with a Perkin-Elmer DSC-7 differential scanning calorimeter/TAC-7 thermal analysis controller with a intracooler-2 cooling system (Perkin-Elmer Instruments, USA). The amount of product to be analyzed shall range from 3 to 5 mg and be placed in perforated aluminium sealed 50 µl pans. Heat runs for each sample has been set from 40 to $200^0$C at $5^0$ C/min, using nitrogen as blanket gas(20 mL/min).

### *Powder X-ray diffraction (PXRD)[21]*

PXRD diffractograms of each of the excipients, and all of the un-milled and milled atorvastatin formulations were recorded using a Siemens Diffractometer D5000 (Siemens, Germany) with Ni-filtered Cu Kα radiation. The $2\theta$ scan range was $5–60^0$. with a step size of $0.02^0$ and the scan speed was $3^0$ min-1.

### *Thermal gravimetric analysis (TGA)*

Thermal gravimetric analysis (TGA) was carried on a TA instruments (USA) TGA 2950 Thermogravimetrical Analyzer over a temperature range of $20–300^0$C at a heating rate of $5^0$C/min under nitrogen flow (50 mL/min). Approximately 5mg of sample was placed in open aluminum pans and the weight loss was monitored.

## RESULTS AND DISCUSSION

### *Characterization of nano particles*

Atorvastatin nanoparticles were obtained by high pressure homogenization. DSC, PXRD, TGA analysis and solid-state properties including solubility, and dissolution were studied to characterize the particles obtained by HPH. The powder X-ray diffraction patterns of unprocessed and processed particles are shown in Fig.

1. Characteristic diffraction peaks were observed for commercial atorvastatin. On the other hand, processed nanoparticles were characterized by the complete absence of any diffraction peak corresponding to crystalline atorvastatin calcium. Fig. 2 shows the DSC curves of commercial drug and the nanoparticles. The DSC curves of commercial atorvastatin shows a broad endotherm ranging from 50 to 130$^0$C indicating the loss of water and the sharp endotherm at 155.96$^0$C might be due to the melting point of atorvastatin calcium. However, no endotherms were seen in the DSC curves for the nanoparticles obtained by HPH. These results indicate that atorvastatin is no longer present as a crystalline form when processed by HPH. The TGA curves of unmilled and HPH processed drug particles are shown in Fig.3. Commercial drug particles exhibited a gradual decrease in weight of about 4.46% due to the loss of water which indicates that the trihydrate form of the drug has been converted to anhydrous form. The SEM images (not shown) shows irregular-shaped crystals for the commercial drug and a drastic change in the morphology and shape of drug was observed for all processed particles.

***Particle size analysis***

Commercial atorvastatin used for this study was characterized by relatively large particles ($d(v; 0.5)$ about 38µm as reported in Table 1 and had to follow preliminary size reduction steps prior to high pressure homogenization operation as the homogenizing gaps of the homogenizer are too small at the homogenizing pressures used (i.e. 25 µm at 22,000 PSI). Table 1 show the results of size analysis following the different size reduction steps and indicate that the low pressure pre-milling homogenization cycles are not sufficient for adequate particle size reduction achievement as they only yield a small percentage of sub-micrometer sized particles. High pressure homogenization cycles were found necessary in that regard; yielding a nanoparticle population with a $d(v; 0.5)$ around 200 nm.

***Saturated solubility studies***

The solubility data of commercial and processed atorvastatin particles are shown in Table 1. In the case of commercial atorvastatin particles, the equilibrium solubility (approximately 142µg/mL) was reached rapidly. In contrast, the maximum supersaturated concentrations of atorvastatin from nanoparticles was about 483µg/mL.

***Dissolution studies***

Dissolution rate enhancement is clearly evident for homogenized product in comparison of unprocessed atorvastatin (Fig.4). Approximately 90% of the atorvastatin was already dissolved after 10 min for nanoparticles, compared to dissolution of below 50% and 25% of premilled drug and commerical atorvastatin respectively[25] after the same time period. The increased dissolution rate can be explained by reduced particles size of nanoparticles. The high pressure homogenization process decreased the size of solid particles to the nanometer scale and simultaneously increased the surface area of particles dramatically. By the increase in surface area, the high energy state achieved will increase the extent to which the particles can dissolve due to an increase in dissolution pressure.

**CONCLUSIONS**

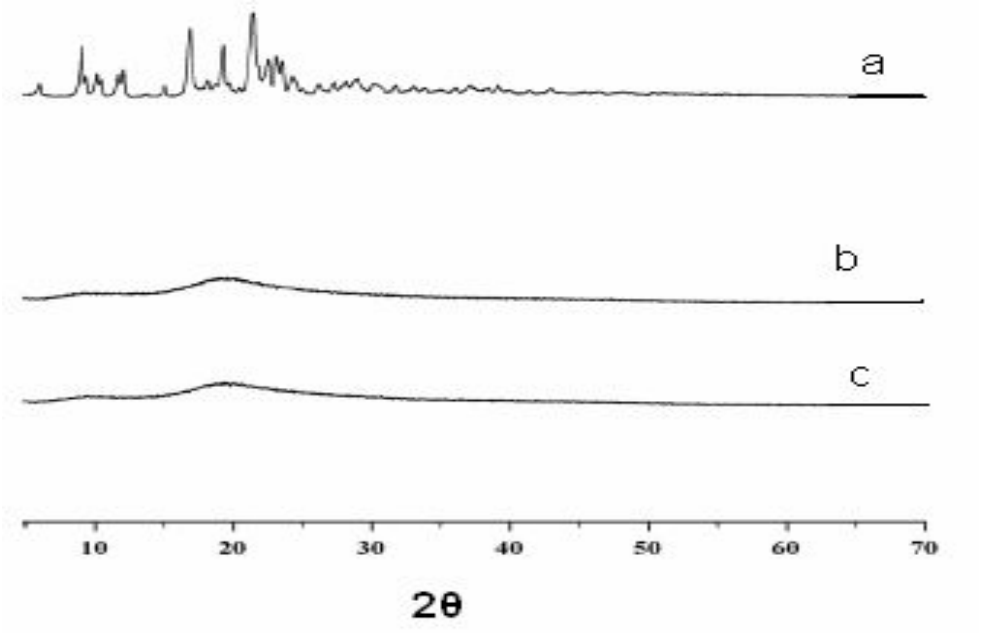
In this study, atorvastatin calcium nanoparticles were successfully prepared by high pressure homogenization and were evaluated for its various solid state characteristics. Through the various studies performed it was found that crystalline atorvastatin was converted to amorphous form and exhibit improved dissolution and higher solubility. The increase in drug dissolution rate and solubility can be expected to have significant impact in the oral bioavailability of the drug. This study shows the effectiveness of the high pressure homogenization technique as a promising approach for enhancing the dissolution of poorly soluble drugs like atorvastatin calcium.

**Table 1: Effect of Milling operation on particle size and solubility**

| Sample | d(υ,0.5)µm | d(υ,0.1) µm | d(υ,0.9) µm | Solubility (µg/ml) |
|---|---|---|---|---|
| Unmilled drug | 38.3±0.06 | 21.9±0.2 | 87.8±0.4 | 142.2 |
| Turrax® milling | 14.2±0.02 | 3.83±0.08 | 24.2±0.5 | 198.3 |
| Pre milling HPH | 2.12±0.08 | 0.486±0.017 | 4.89±0.25 | 322.5 |
| HPH(20cycles @1500bar) | 0.241±0.06 | 0.068±0.003 | 0.964±0.02 | 483.2 |

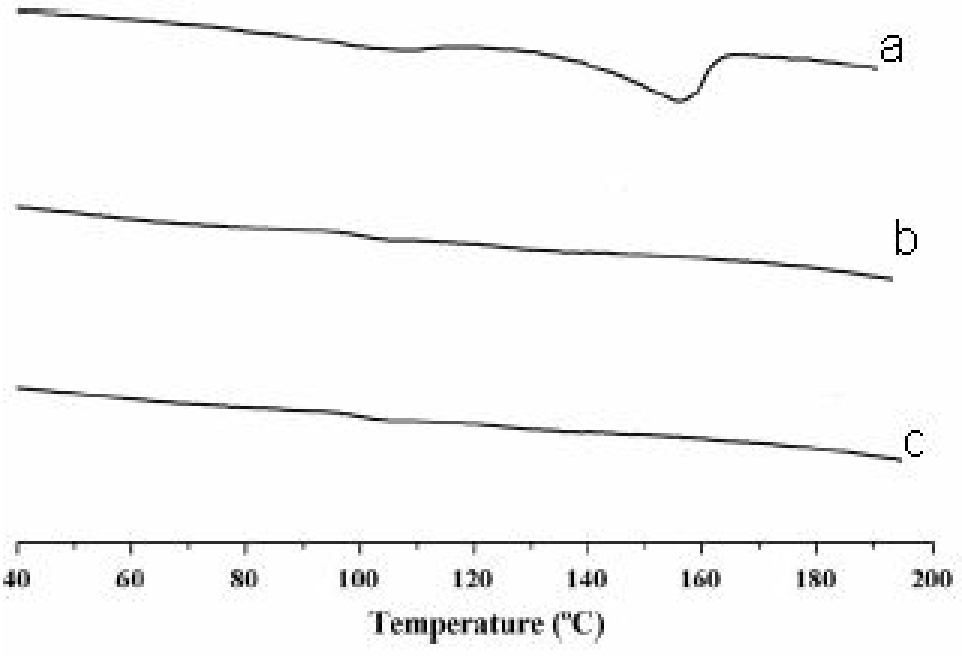
(HPH - High pressure homogenization)

**Figure 1: XRD patterns**



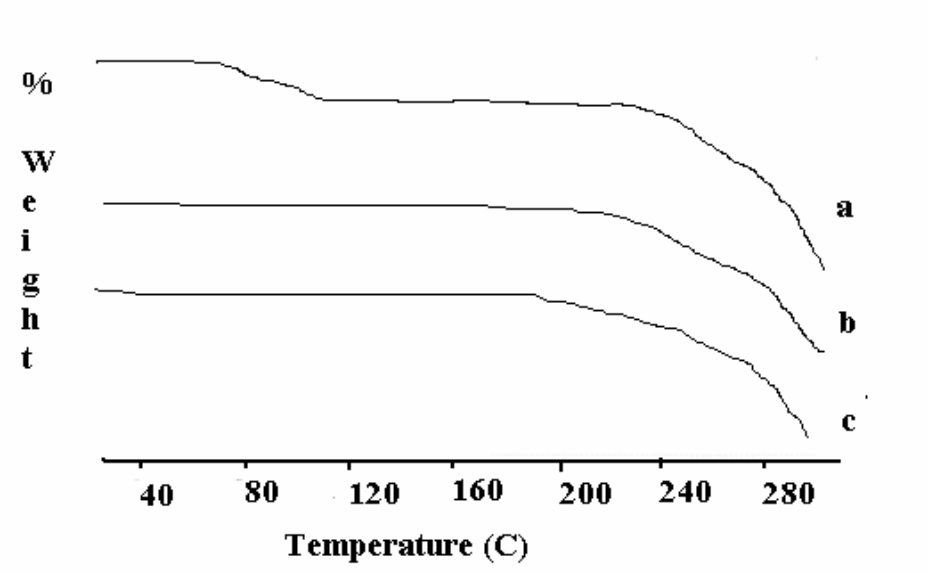

(a) commercial drug (b) premilled drug (c) HPH processed drug

**Figure 2: DSC Thermograms**



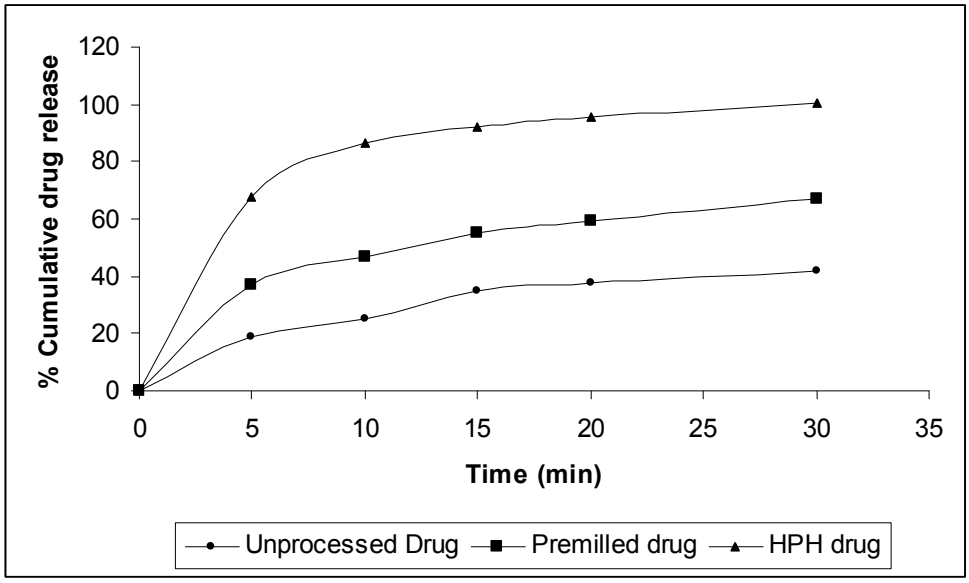

(a) commercial drug (b) premilled drug (c) HPH processed drug

**Figure 3: TGA Thermograms**




(a) commercial drug (b) premilled drug (c) HPH processed drug

**Figure 4: Dissolution Profile of samples**

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