

# Formulation development and *in vitro* evaluation of nanosuspensions loaded with Atorvastatin calcium

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The aim of this study was to prepare and characterize nanosuspensions of a poorly soluble drug (Atorvastatin calcium) in order to enhance its solubility and dissolution characteristics. Nanosuspensions were prepared by high pressure homogenization technique. They were characterized by thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), solubility, and *in vitro* drug release studies. The absence of atorvastatin peaks in PXRD profiles of nanosuspensions suggests the transformation of crystalline drug into an amorphous form. TGA examination suggested that the drug was converted into anhydrous form from the original trihydrate form. DSC curves also compliment the result obtained by TGA and PXRD. The effect of particle size was found to be significant on the saturation solubility of the drug. The *in vitro* drug release studies showed a significant increase in the dissolution rate of nanosuspensions as compared with pure drug. This study has shown that initial crystalline state is reduced following particle size reduction and that the dissolution characteristics of atorvastatin nanosuspensions were significantly increased in regards to the pure drug. The method being simple and easily scaled up, this approach should have a general applicability to many poorly water-soluble drug entities.

**Key words:** Atorvastatin, crystalline state, dissolution, high pressure homogenization, nanosuspensions

## INTRODUCTION

A major hurdle that has prevented the commercialization of many promising poorly soluble drug candidates is dissolution rate-limited bioavailability. Compounds exhibiting dissolution rate-limited bioavailability are considered class II according to the BCS classification.<sup>[1]</sup> As per recent report,<sup>[2]</sup> 46% of the total NDAs filed between 1995 and 2002 were BCS class IV, while only 9% were BCS class I drugs, revealing that a majority of approved new drugs were water insoluble. There are drug candidates that have poor solubility in water but can be dissolved by suitable conventional formulation strategies which include co-solvents,<sup>[3]</sup> milling techniques,<sup>[4]</sup> super critical processing<sup>[5]</sup> and solid dispersions<sup>[6]</sup> including complexation<sup>[7]</sup> and precipitation techniques.<sup>[8]</sup> However, there still remains an unmet need to equip pharmaceutical industry with particle engineering technologies capable of enhancing

the dissolution of poorly soluble compounds. One such novel technology is Nanosuspension technology.

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.<sup>[9]</sup> The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH. The drug is absorbed more in the upper duodenum and in the upper small intestine regions. However, it is reported that the absolute bioavailability (*F*) of atorvastatin is 12% after a 40 mg oral dose.<sup>[10]</sup> In the present study, an attempt was made to enhance the solubility and dissolution characteristics of a poorly soluble model drug, atorvastatin calcium (AC) using nanosuspension technology.

## MATERIALS AND METHODS

AC was obtained as a gift sample from M/s. Caplin point, Pondicherry, India. Polyvinyl pyrrolidone was a gift sample from M/s. Colorcon, Goa, India. All other chemicals and solvents used are of analytical grade.

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### Preparation of nanosuspensions

Atorvastatin powder (5%w/v) was dispersed in an aqueous surfactant solution (0.2% w/v, suspensions) under magnetic stirring (1000 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 and the obtained mixture was homogenized at room temperature using a Micron LAB 40 (APV Systems, Unna, Germany). At first, 2 cycles at 100 bar and 2 cycles at 500 bar as premilling steps were applied, then 20 cycles at 1500 bar were run to obtain the nanosuspension.<sup>[11]</sup> Samples were withdrawn at each size reduction steps for size distribution analysis.

### Production of dry nanoparticles

Spray-drying using a Büchi B191a Mini Spray- Dryer (Büchi, Flawil, Switzerland) was applied in order to retrieve nanoparticles in dried-powder state from the nanosuspensions described above. Suspensions were passed at a spray rate of 3.5 ml/min for 30 min. The drying temperature was set at 115°C. Spray airflow was set at 800 l/h and drying airflow was set at 35 m<sup>3</sup>/h.

### Particle size analysis

The particle size analysis was performed laser diffractometry (LD) using the Mastersizer E (Malvern Instruments). The LD yields a volume distribution. The particle size,  $d(v; 0.5)$  (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. Before measurement, the samples had to be diluted with deionized water to obtain a suitable concentration for measurement.

### Zeta potential analysis

Zeta potential analysis was performed to estimate the stability of the nanosuspensions using Malvern Zetasizer 4 (Malvern Instruments). The samples were diluted with deionized water with conductivity adjusted to 50  $\mu\text{S}/\text{cm}^2$  by addition of sodium chloride before measurement. All measurements were performed in triplicate.

### *In vitro* drug release studies

*In vitro* drug release studies were performed in an USP Type II dissolution apparatus (Electro lab, India) using paddle method at rotation speed of 50 rpm. Dissolution was carried out both in acid media and neutral media using an equivalent of 10 mg of atorvastatin. The volume and temperature of the dissolution medium were 900 ml and  $37.0 \pm 0.2$  °C, respectively. Samples were withdrawn at fixed times and were filtered and assayed through ultraviolet absorbance determination at 245 nm using a Shimadzu UV-Visible spectrophotometer. The mean results of triplicate measurements and the standard deviation were reported. The results obtained were compared with the dissolution profile of marketed preparation.

### Saturation solubility studies

Saturation solubility measurements were assayed through ultraviolet absorbance determination at 245 nm using a Shimadzu UV-Visible 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 Hecq *et al.*<sup>[11]</sup> 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 37°C and equilibrated for 2 days. An aliquot was passed through 0.1  $\mu\text{m}$  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.

### Wettability study

The pure drug and formulations were subjected to wettability studies by the Buchner funnel method<sup>[12]</sup> and water absorption method.<sup>[13]</sup> In the first method, the pure drug and formulations of about 100 mg were weighed and placed in a Buchner glass funnel. The funnel was plunged into a beaker containing water in a manner that the beaker remains at the same level as the powder in the funnel. Methylene blue powder (100 mg) was layered uniformly on the surface of the powder in the funnel. The time required for wetting methylene blue powder was measured.

In the water absorption method, a tissue paper was placed in a petri dish of 10 cm diameter. Methylene blue, a water soluble dye, was added to the petri dish. The dye solution was used to identify complete wetting of the tablet surface. A tablet prepared with pure drug and the formulations was carefully placed on the surface of the tissue paper in the petri dish. The time required for water to reach the upper surface of the tablets and to completely wet them was taken as the wetting time. The weight of the tablet prior to placement in the petri dish was noted ( $w_b$ ), the wetted tablet was removed and reweighed ( $w_a$ ), water absorption ratio  $R$  was then determined according to the following equation:

$$R = 100 * (w_a - w_b) / w_b$$

### Permeation study

The permeation study of the pure drug, pre-milled drug, and HPH processed drug was carried out using two different membranes, namely egg membrane<sup>[14]</sup> and cellulose nitrate membrane.<sup>[15]</sup> The diffusion of the drug through the membranes was analyzed in a diffusion cell. The required length of membrane was cut and attached to the ground bottom layer of the diffusion cell with glue. The cell (donor compartment) was marked for its 10 ml content and was dipped inside a beaker (receptor compartment) containing phosphate buffer pH 6.8. 10 ml of the buffer was added to the donor compartment. It was noted the level of the buffer

inside the cell and in beaker was of same level. Weighed amount of the pure drug and formulations equivalent to specified quantity of the drug was added to the cell. The samples at predetermined time intervals were withdrawn and the same volume of fresh buffer was replaced immediately to maintain sink condition. The study was carried out for 1 h. The solutions were suitably diluted and the absorbances were measured using UV spectrophotometer at 245 nm.

### Solid state characterization

#### Thermal gravimetric analysis

Thermal gravimetric analysis (TGA) was carried on a TA instruments (USA) TGA 2950 Thermo gravimetric Analyzer over a temperature range of 20–300°C at a heating rate of 5°C/min under nitrogen flow (50 ml/min). Approximately 5 mg of sample was placed in open aluminum pans and the weight loss was monitored.<sup>[16]</sup>

#### Differential scanning calorimetry

The thermal properties of the powder samples were investigated with a Perkin-Elmer differential scanning calorimetry (DSC)-7 differential scanning calorimeter/TAC-7 thermal analysis controller with an 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°C at 5°C/min, using nitrogen as blanket gas (20 ml/min).<sup>[17]</sup>

#### Powder X-ray diffraction

Powder X-ray diffraction (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 K $\alpha$  radiation. The 2 $\theta$  scan range was 5–60° with a step size of 0.02° and the scan speed was 3° per min.<sup>[18]</sup>

## RESULTS

### Solid state evaluation of nano particles

In this study, an attempt was made to prepare amorphous atorvastatin particles by high pressure homogenization (HPH) technique. TGA, DSC, PXRD analysis, solubility, and *in vitro* release were studied to characterize the particles obtained by HPH. The TGA curves of commercial and drug nano particles are shown in Figure 1. Theoretically, the stoichiometric value of the trihydrate should be 4.46%. Commercial particles exhibited a gradual decrease in weight about 4.46% due to the loss of water. Figure 2 shows the DSC curves of commercial, premilled, and HPH processed particles. The powder X-ray diffraction patterns of commercial and processed particles are shown in Figure 3. 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 AC.

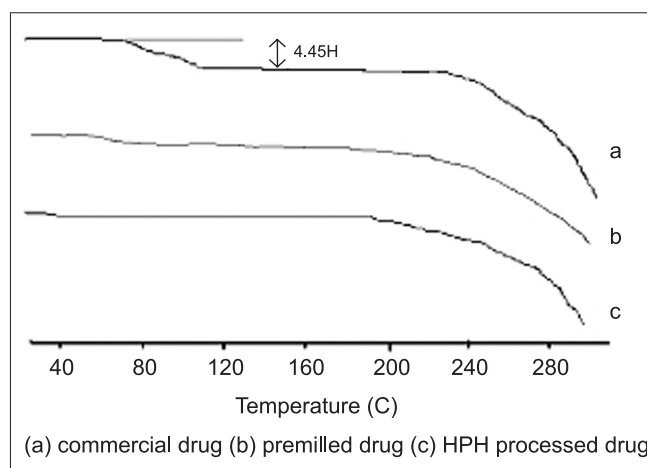


Figure 1: TGA thermograms

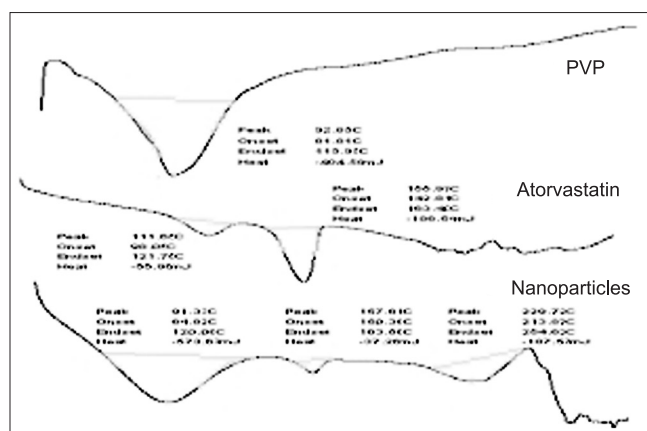


Figure 2: DSC thermograms

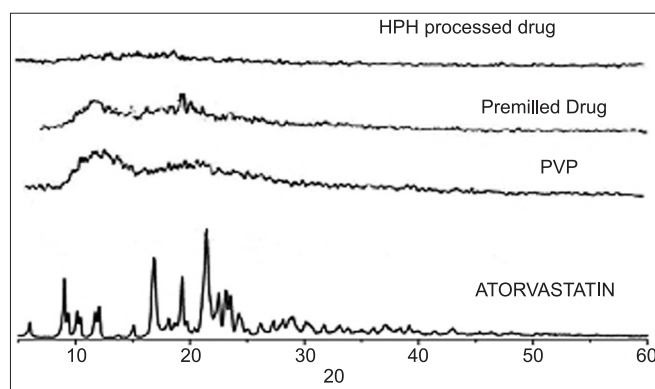


Figure 3: XRD patterns

### Particle size analysis

Commercial atorvastatin used for this study was characterized by relatively large particles ( $d(v; 0.5)$ ) about 38 µm 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: Effect of milling operation on particle size and solubility**

Sample	d(u,0.5) $\mu\text{m}$	d(u,0.1) $\mu\text{m}$	d(u,0.9) $\mu\text{m}$	Solubility ( $\mu\text{g/ml}$ )
Commercial drug	38.3 $\pm$ 0.06	21.9 $\pm$ 0.2	87.8 $\pm$ 0.4	142.2 $\pm$ 0.5
Turrax <sup>®</sup> milling	21.5 $\pm$ 0.03	5.21 $\pm$ 0.06	32.2 $\pm$ 0.5	185.1 $\pm$ 1.2
Pre milling HPH	3.12 $\pm$ 0.05	0.79 $\pm$ 0.01	8.11 $\pm$ 0.2	299.8 $\pm$ 0.6
HPH(20cycles@1500bar)	0.446 $\pm$ 0.02	0.182 $\pm$ 0.03	1.112 $\pm$ 0.03	386.5 $\pm$ 0.7

(HPH - High pressure homogenization,  $\pm$  S.D, n=3)

**Table 2: Permeability and wettability data**

Formulation	Permeability (mg/ml/h)		Wettability	
	Egg membrane	Cellulose nitrate membrane	Buchner Funnel Method (min)	Water absorption method (R)
Pure drug	0.0030	0.0137	80 $\pm$ 0.5	3.904 $\pm$ 0.002
Pre-milled drug	0.0079	0.0284	42 $\pm$ 0.3	14.40 $\pm$ 0.01
HPH processed drug	0.0103	0.0328	30 $\pm$ 0.2	18.92 $\pm$ 0.03

### Zeta potential analysis

Zeta potential values give a measure of the long-term stability for the particulate systems. For a physically stable suspension stabilized by electrostatic repulsion, a zeta potential of about  $\pm 30$  mV is required as minimum. In a combined electrostatic and steric stabilization, a minimum of  $\pm 20$  mV will be sufficient.<sup>[19]</sup> The investigated nanosuspension showed a value of about  $\pm 33$  mV which indicates good stability of the prepared formulation.

### Saturated solubility studies

The solubility data of unprocessed and processed atorvastatin particles are shown in Table 1. In the case of commercial atorvastatin particles, the equilibrium solubility (approximately 142  $\mu\text{g/ml}$ ) was reached rapidly. In contrast, the maximum supersaturated concentration of atorvastatin from nanoparticles was about 386.5  $\mu\text{g/ml}$ .

### Wettability studies

The Buchner funnel method and water absorption method for finding out wettability were investigated as per the method reported by M. C. Gohel *et al.*<sup>[12]</sup> and Sunil Kumar *et al.*<sup>[13]</sup> respectively and findings are shown in Table 2. The wetting time and water absorption ratio of the pure drug was found to be 80 min and 3.904, respectively, indicating its poor wettability. The wetting time of pre-milled drug and HPH processed drug was found to be very less (42 and 30 min) and water absorption ratio was more (14.40 and 18.92) than pure drug.

### Permeation study

The permeation study through egg membrane was done as per the method reported by Mehdi Ansari *et al.*<sup>[14]</sup> and Giovanna Corti *et al.*<sup>[15]</sup> The data for egg membrane and cellulose nitrate membrane was given in Table 2. It was observed that the amount of drug permeated in both membranes was found to be higher for the pre-milled drug

and HPH processed drug than the pure drug. Permeation through cellulose nitrate membrane shows better result when compared with egg membrane. The results can be considered as the evident for increase in release rate of the drug.

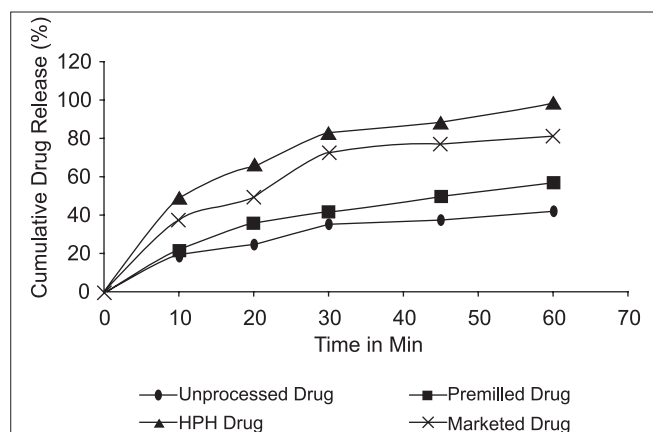
### Dissolution studies

Dissolution rate enhancement is clearly observed for homogenized product in comparison of unprocessed atorvastatin. Dissolution profiles in acid and neutral media for unprocessed and processed particles are shown in Figures 4 and 5. On comparing the dissolution parameters, it was noticed that HPH processed drug showed a higher cumulative release (98.4% in neutral media and 45.6% in acid media), better dissolution efficiency, and less mean dissolution time than the marketed drug (81.22% and 22.1%), pre-milled drug (57.2% and 28.3%), and the pure drug (42.08% and 10.25%).

## DISCUSSIONS

The TGA results showed a weight loss of 4.46% for the commercial drug. The total weight loss corresponding to a water loss of about 4.46% is in agreement with stoichiometric value of a trihydrate. On the other hand, this weight loss was not seen for all processed particles. From these TGA results, it can be seen that commercial AC trihydrate was transformed to anhydrous form by the HPH process. In DSC curves of unprocessed atorvastatin, a broad endotherm ranging from 90 to 130°C indicating the loss of water and a sharp endotherm at 155.97°C might be due to the melting point of AC. However, no sharp endotherms were observed 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, but exists in the amorphous state. The energy requirement for the pure drug was about 188.54 mJ, whereas the HPH processed drug required only 37.26 mJ which shows the reduction in crystallinity of the pure drug. The saturation solubility





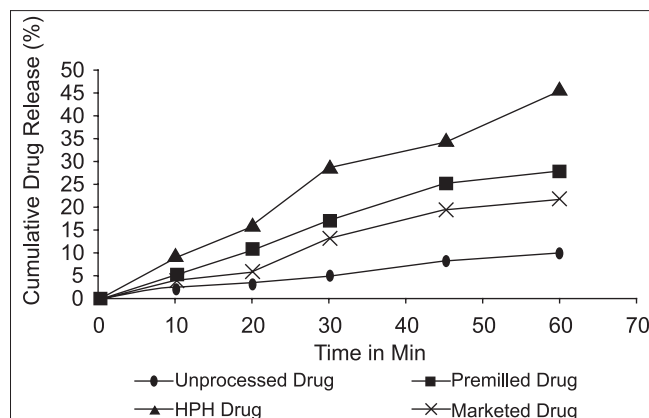
**Figure 4:** Dissolution profile in neutral media

results obtained in the study was in correlation with the results already reported by Kim *et al.*<sup>[20]</sup> It is suggested that these dissolution behaviors of all processed particles might be due to the amorphous nature.

Table 1 shows the results of size analysis following the different size reduction steps and indicates 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 450 nm. The zeta potential analysis which is indicative of the stability of the prepared nanosuspensions showed a value of about  $-33$  mV which shows that the suspension will be stable enough for a long term. Table 2 shows the permeability and wettability data of the pure drug in comparison with the pre-milled drug and the HPH processed drug. It was clear from the results that the permeability of the drug and the wettability has been increased significantly. This increase may lead to improved absorption *in vivo*. The dissolution profiles obtained in the study clearly gives indication about the enhancement of solubility of the pure drug by using this technology. The HPH processed drug showed a cumulative release of about 98.4% at the end of 1 h, whereas the cumulative release of the pure drug and the marketed sample was only about 42% and 81%, respectively. 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 significantly which led to the increase in the dissolution.

## CONCLUSIONS

In this study, AC nanosuspensions were successfully prepared by high pressure homogenization and were evaluated for its physicochemical properties. The physicochemical characterization showed that crystalline atorvastatin was



**Figure 5:** Dissolution profile in acid media

converted to amorphous form and exhibited enhanced dissolution rate and high saturation solubility due to its amorphous nature, in comparison with crystalline atorvastatin. The increase in drug dissolution rate and solubility can be expected to have a significant impact on the oral bioavailability of the drug. This study demonstrated the usefulness of the high pressure homogenization technique as a method of enhancing the dissolution of poorly soluble drug-like AC.

## REFERENCES

1. The Bio pharmaceuticals Classification System (BCS) Guidance, Office of Pharmaceutical Science. Available from: [http://www.fda.gov/cder/OPS/BCS\\_guidance.htm](http://www.fda.gov/cder/OPS/BCS_guidance.htm). [last accessed on 2008 Jan 12].
2. Clewlow PJ. Survival of the smartest: Scrip's target world drug delivery news. Informa Healthcare, UK 2004;35:316-23.
3. Seedher N, Kaur J. Solubilization of nimesulide: Use of co-solvents. Indian J Pharm Sci 2003;65:58-61.
4. Mersiko-Liversidge E, MGurk SL, Liversidge GG. Insulin nanoparticles: A novel formulation approach for poorly water soluble Zn-Insulin. Pharm Res 2004;21:1545-53.
5. Benjamin C, Lu Y, Zang D, Sheng W. Solubility enhancement in supercritical fluids. Pure Appl Chem 1990;62:2277-85.
6. Paradkar A, Ambike A, Jadhav B, Mahadik K. Characterization of curcumin-PVP solid dispersion obtained by spray drying. Int J Pharm 2004;271:281-6.
7. Anzai K, Mizoguchi J, Yanagi T, Hirayama F, Arima H, Uekama K. Improvement of dissolution properties of a new Helicobacter pylori eradicating agent (TG44) by inclusion complexation with beta-cyclodextrin. Chem Pharm Bull 2007;55:1466-70.
8. Sucker H. Hydrosol, eine Alternative für die parenterale Anwendung von schwer wasserlöslichen Wirkstoffen. In: Muller RH, Hildebrand GE, editors. Pharmazeutische Technologie; Modern Arzneiformen. 2<sup>nd</sup> ed. Stuttgart: WVG; 1998.
9. Lennernas H. Clinical pharmacokinetics of atorvastatin. Clin Pharmacokinet 2003;42:1141-60.
10. Corsini A, Bellosa S, Baetta R, Fumagalli R, Paoletti R, Bernini F. New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther 1999;84:413-28.
11. Hecq J, Deleers M, Fanara D, Vrandex H, Amighi K. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine. Int J Pharm 2005;299:167-77.
12. Gohel MC, Patel LD. Processing of Nimesulide-PEG 400-PEG-PVP solid dispersions: Preparation, characterization and *in vitro* dissolution. Drug Dev Ind Pharm 2003;29:299-310.

13. Sunil KB, Michael AR, Soumyajit M, Rao Y. Formulation and evaluation of rapidly disintegrating Fenoverine tablets: Effect of superdisintegrant. *Drug Dev Ind Pharm* 2007;33:1225-32.
14. Mehdi A, Maryam K, Monireh A. The study of drug permeation through natural membrane. *Int J Pharm* 2006;327:6-11.
15. Giovanna C, Francesca M, Marzia C, Sandra F, Paola M. Development and evaluation of an *in vitro* method for prediction of human drug absorption 1: Assessment of artificial membrane composition. *Eur J Pharm Sci* 2006;27:346-53.
16. Kim MS, Jin SJ, Kim JS, Park HJ, Song HS, Neubertb RH, *et al.* Preparation, characterization and *in vivo* evaluation of amorphous atorvastatin calcium nanoparticles using supercritical antisolvent (SAS) process. *Eur J Pharm Biopharm* 2008;69:454-65.
17. Kocbek P, Baumgartner S, Kristi J. Preparation and evaluation of nanosuspensions for enhancing dissolution of poorly soluble drugs. *Int J Pharm* 2006;312:179-86.
18. Yuichi T, Atsutoshi I, Hihiroko S, Toshio O, Keiji Y. Characterization and quantitation of Clarithromycin polymorphs by powder X-Ray diffractometry and solid state NMR spectroscopy. *Chem Pharm Bull* 2002;50:1128-30.
19. Jacobs C, Muller RH. Production and characterization of budesonide nanosuspensions for pulmonary administration. *Pharm Res* 2002;19:189-94.
20. Kim JS, Kim MS, Park HJ, Jin SJ, Sibeum L, Hwang SJ. Physicochemical properties and oral bioavailability of amorphous atrovastatin hemicalcium using spray-drying and SAS process. *Int J Pharm* 2008; 359:211-9.

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