Design, Development, and Characterization of Atorvastatin Calcium Nanocrystal for Improved Oral Bioavailability

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Abstract:

The objective of the study was to elevate the solubility, dissolution, and oral bioavailability of a practically insoluble hyperlipidemia drug Atorvastatin calcium. 2^3 factorial design and solvent antisolvent precipitation were used to create atorvastatin calcium nanocrystals (NCs). Particle size, drug content, surface morphology, saturation solubility, zeta potential, and in vitro release studies were all assessed in a variety of trials. Three parameters i.e., stabilizer (Pluronic F-127) concentration, solvent antisolvent ratio, and stirring time were examined for their effects on NCs characteristics. FTIR studies indicate no drug-polymer interactions. NCs had a zeta potential value of -38.2 mv with particle sizes varying from 128 to 316 nm. SEM was utilized to investigate the surface morphology of NCs, which showed a typical spherical structure with a uniform surface free of collapse. The drug content of NCs varied from 50 to 78. When compared to the pure drug, the optimized formulation NCs demonstrated a significant improvement in saturation solubility and a quick rate of dissolution. The production of Atorvastatin calcium NCs using solvent antisolvent precipitation technique could enhance the drug's solubility, dissolution, and bioavailability, which may solve the issues related to traditional formulations such as tablets.

Keywords: Atorvastatin Calcium, Nanocrystals, Solvent Antisolvent Precipitation Method, and Bioavailability.

1. Introduction:

One of the biggest obstacles to drug delivery is the low water solubility and low bioavailability of novel chemical entities. Recent years have shown that about 40% of newly licensed oral immediate-release medications and 70% of newly developed medications have limited water solubility [1]. To enhance the drug's solubility and dissolution, pharmaceutical researchers are looking into a variety of approaches such as solid dispersions, salt formation, micronization, co-crystallization, self-emulsification, solid lipid nanoparticles, nanocrystallization, cyclodextrin inclusions. Among these, nanocrystallization has gained interest as a successful method of delivering poorly soluble drugs.

Nanocrystallization techniques involve bottom-up as well as top-down approaches. In the top-down approach, large particles are reduced to the desired size range, whereas in the bottom-up approach, particles are created from solutions by adding antisolvent. The top-down strategy uses high-pressure homogenization, which produces nanosuspension that can be dried further by freeze or spray drying. The bottom-up

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H. Roy et al. (eds.), *Proceedings of the International Symposium on Sustainable Drug Design and Nanoparticle Development: Quantum and Computational Perspectives (SDDNDQCP 2025)*, Advances in Health Sciences Research 89,
https://doi.org/10.2991/978-94-6463-813-4_8

strategy entails adding a drug solution to an antisolvent to induce controlled precipitation. Choosing an appropriate solvent and antisolvent system is crucial for producing an optimal formulation with the required characteristics. Nanocrystals can be manufactured by these two approaches.

Atorvastatin calcium (ASC) is an anti-hyperlipidemic drug. Atorvastatin inhibits the enzyme HMG-CoA reductase. Atorvastatin is used to reduce blood cholesterol and triglycerides. It belongs to the BCS II drug and has lower solubility and bioavailability [2]. Although it achieves rapid absorption when administered orally due to intestinal and first-pass metabolism its bioavailability is low. The major problem with ASC is instability and poor solubility [3]. Hence increasing its solubility, bioavailability, and decreasing cost and associated side effects is crucial.

ASC was opted as the model drug for this investigation because it is a drug that is only marginally soluble in water, making it a perfect choice to explore the potential of rapid-release nanocrystals. The study aimed to increase the dissolution and bioavailability of ASC by developing nanocrystals using the solvent antisolvent precipitation method. Acetone is used as a solvent, and Pluronic F-127 is used as an antisolvent and stabilizer.

2. Materials and Methods:

2.1. Materials: Atorvastatin Calcium is purchased from Yarrow Chem Products (Mumbai), Acetone, and Sodium hydroxide buffering agent acquired from S.D. Fine Chem. Ltd (Mumbai), Pluronic F-127 Sigma-Aldrich (Bangalore), Potassium Dihydrogen Phosphate buffering agent from Qualigens Fine chemicals.

2.2. Methods:

2.2.1. Pre-formulation studies: Pre-formulation is a subset of research focusing on a novel drug candidate's physicochemical attribute that could affect the drug's effectiveness and dosage form design. So, before developing a dosage form the physicochemical properties of the drug are evaluated [4].

2.2.1.1. Standard Curve of ASC: To make PB with a pH of 6.8, 23.65 ml of 0.2 M NaOH was added to 50 ml of 0.2 M KH_2PO_4, and then 200 ml of distilled water was used to dilute the mixture. The 100 mg ASC was dissolved in 100 ml of pH 6.8 PB to get a 1000 µg/ml standard solution, and 1 ml of this was diluted with 100 ml of pH 6.8 PB to get a 10 µg/ml solution. To make working solutions with concentrations of 1, 3, 5, 7, and 9 µg/ml, portions of the standard solution were pipetted and then diluted with pH 6.8 PB. A calibration curve was created by measuring the absorbance at 242 nm [6].

2.2.1.2. Drug-Excipient Compatibility Studies: Excipients and their concentration in a formulation is based not only on their functionality but also on the compatibility between the drug and excipient. Although most of the excipients have no direct pharmacological action these are important for prolonging the stability of the drug, enhancing bioavailability, patient compliance, and masking the bitter taste of the drug. So, detecting the compatibility between the drug and the excipient is crucial. Compatibility studies were carried out by following the KBR pellet method using the FTIR spectrophotometer (Bruker) [7].

2.3. Formulation development:

2.3.1. 2^3Factorial design: The optimization phase was designed (shown in Table 1 and 2) using a 2^3 factorial design in which three variables namely concentrations of stabilizer, solvent antisolvent ratio, and stirring time were maintained at two levels.

2.4. Preparation:

2.4.1. Solvent Antisolvent Precipitation Method: In this approach, the solvent mixture is added to the antisolvent mixture at a predetermined rate. A solvent mixture is prepared by dissolving the drug (10mg) in acetone (2ml). This mixture is added at a predetermined rate (0.2ml/min or 0.6 ml/min) to the antisolvent mixture containing Pluronic F-127 (stabilizer) and water under continuous stirring (Remi) at a predetermined time (15 min or 30 min) and speed (2000rpm). The formed nanosuspension is freeze-dried to obtain nanocrystals of ASC [8]. The whole process is depicted in Figure 1.

2.4.2. Characterization of nanocrystals:

2.4.2.1. Particle Size and Size Distribution Analysis: Particle size was analyzed by Zetasizer® 3000 (Malvern Instruments, Sura labs, Hyderabad). Every sample was diluted with ultra-purified water, and measurements were made for 180 seconds at 25° and 90° scattering angles. Each sample's mean diameter was produced in triplicate using cumulative analysis [9]. The size distribution was given by the polydispersity index and the data reported represent the average of 3 measurements.

2.4.2.2. Scanning electron microscopy: The morphological examination of NCs was done by SEM (Tecnai 20 G2 S TWIN at Sura Labs, Hyderabad). From a structural perspective, the behavior and stability of nanocrystals can be determined by the orientation of molecules and the arrangement of components [10].

2.4.2.3. Zeta potential: To measure the zeta potential (ζ), a Zeta Sizer (Sura labs, Hyderabad) was used. Carried out by inserting diluted samples into the capillary measuring cell and adjusting the cell position [11]. The surface potential of the particles is essential in maintaining stability. The optimum potential is essential for uniformity in dispersion and avoiding caking and other effects.

2.4.2.4. Drug Content: The drug content was found using the centrifugation method. To isolate the free drug, the NCs suspension was centrifuged for 45 minutes at 4°C and 20,000 rpm. Following appropriate dilution, the concentration of ASC in the supernatant was measured using a UVS at 242 nm [12].

2.4.2.5. Saturation Solubility: Saturation solubility was carried out in a rotary shaker bath at 37±0.5°C. The pure drug and produced NCs, equivalent to 10mg of the medication, were introduced separately into the PB (pH 6.8). After 48 hours, 500 µl of the sample was taken and ultracentrifuged for 15 min at 4°C and 20,000 rpm. To determine the saturation solubility of ASC, the supernatant was appropriately diluted and examined using a UVS.

2.4.2.6. In vitro Dissolution studies: The drug release patterns of Pure drug and prepared nanocrystals (equivalent to 10mg of Atorvastatin Calcium) were compared. Dissolution was carried out under the following conditions:
Apparatus: USP dissolution apparatus II (Lab India Pvt., Ltd., India)
Dissolution medium: pH 6.8 PB

Volume of medium: 900 ml
Speed: 50 rpm
Temperature: 37° ± 1.0°C
Sampling points: 10, 20, 30, 40, 50, and 60 min
Analysis: UV-visible double-beam spectrophotometer at 242 nm [8]

2.4.2.7. Drug release kinetics: The following mathematical models were used to study release kinetics
Zero order equation:

$$Q = Q_0 - k_0 t$$

First-order equation:

$$\ln Q = \ln Q_0 - kt$$

Where Q is the concentration at time t
Q_0 is the initial concentration
k_0 is the zero-order rate constant
k is the first-order rate constant

3. Results and Discussion:

3.1. Standard Curve of ASC: A UVS was used to measure the absorbances of the solutions at 242 nm. A standard graph concentration Vs absorbance was plotted (depicted in Figure 4).

3.2. Drug-Excipient Compatibility Studies: The drug and excipients' spectra revealed a broad peak at the same location as the peak seen in the spectrum of pure ASC (shown in Table 4, Figure 2, and 5), confirming that there was no chemical interaction.

3.3. Characterization of prepared NCs:

3.3.1. Particle Size and Size Distribution Analysis: From table 2 and 3, formulations F1 and F8 demonstrated a maximum particle size of 316 nm and 128 nm, respectively. The results (indicated in Table 5 and Figure 6) specify that the particle size decreased with increasing stabilizer (Pluronic F-127) concentration from 5-10 % and the particle size reduced with increasing solvent antisolvent ratio from 1:5-1:10. The particle size of NCs was also decreased with increasing stirring time from 15-30 min. When the percentage varied in Pluronic F-127 (5-10 %) increased, reduces the interfacial tension, which resulted in significantly increased shear stress during solvent/ antisolvent interface mixing and the resultant formation of smaller droplets. Thus, the mean diameter of NCs decreased with the presence and increase of Pluronic F127.

3.3.2. Scanning Electron Microscopy (SEM): The surface morphology of Pluronic F-127 coated Atorvastatin calcium NCs was evaluated by SEM. The surface morphology of Eight formulations of NCs has a regular spherical shape with a uniform and smooth surface without signs of collapse in Figure 7.

3.3.3. Zeta potential: The zeta potential of formulation F8 was found to be - 38.2, which implies that it has good stability in Figure 8.

3.3.4. Drug content: Table 6, it was found that the F3 formulation had the highest drug content (78 ± 1.36). As the concentration of Pluronic F-127 increased from 5–

10%, the amount of drug was also increased. It decreased with an increased solvent antisolvent ratio from 1:5 to 1:10 and significantly reduced with increasing stirring time. The values are shown in Figure 9.

3.3.5. Saturation solubility: The saturation solubility of NCs lies between 88.9 – 55.3 µg/ml and the pure drug has a saturation solubility of 50.0 µg/ml in a PB solution of pH 6.8. When compared to the pure drug, the improved NCs formulation's saturation solubility demonstrated a notable improvement in solubility. The values are shown in Figure 10.

3.3.6. In vitro Dissolution studies: The prepared NCs showed an increased rate and extent of dissolution. After 60 min of dissolution, 98.53% of the drug was liberated from the NCs compared to 44.7% from the pure drug. Within 10 min, 54.23% of F8 NCs were dissolved in the PB (pH 6.8), while only 15.56% of the pure drug was dissolved in Figure 11 and 12.

3.3.7. Drug release kinetics: Regression analysis was performed on the dissolution data of the pure drug and the improved formulation F8, and the results were fitted to Zero and First order kinetic models (depicted in Figure 13 and 14). The R^2 value for the pure drug for the first order is 0.9852 which is greater than 0.9666 for zero order implying that the drug release followed the first-order mechanism in the case of Atorvastatin Calcium pure, similarly, the R^2 Value of the F8 NCs showed 0.9168 for first order whereas 0.8286 for zero order shows that the F8 NCs also followed first-order mechanism from table 7, 8, and 9.

Table 1: Statistical Design

S. No	Type	Factors (3)	Levels (2)	
			Low level (-1)	High Level (+1)
1	Formulation parameter	Stabilizer	5%	10%
		Solvent:Antisolvent ratio	1:5	1:10
2	Critical process parameter	Stirring time	15 min	30 min

Table 2: Formulation of Nanocrystals

Formulation	Stirring time (min)	Stabilizer concentration	Solvent:Antisolventratio
F1	15	5%	1:5
F2	30	5%	1:5
F3	15	10%	1:5
F4	30	10%	1:5
F5	15	5%	1:10
F6	30	5%	1:10
F7	15	10%	1:10
F8	30	10%	1:10

Table 3: Standard Curve of ASC

Concentration (µg/ml)	Absorbance (242 nm)
0	0.0
1	0.048
3	0.155
5	0.239
7	0.337
9	0.486

Table 4: Characteristic Peaks Observed in FTIR Spectrum

Type of peak	Characteristic value	Pure	Formulation
C-F- stretching	691.35	Yes	Yes
-OH bending	1159.08 cm^{-1}	Yes	Yes
C-N – Stretching	1316.80cm^{-1}	Yes	Yes
-C=O stretching	1650.71 cm^{-1}	Yes	Yes
C=C – bending	1650.71 cm^{-1}	Yes	Yes
N-H – stretching	3152.09 cm^{-1}	Yes	Yes

Table 5: Mean Particle Size of NCs (F1 – F8)

S. No	Formulation	Mean particle Size (nm)	Polydispersity
1	F1	316 ± 1.32	0.51 ± 0.14
2	F2	297 ± 5.24	0.43 ± 0.12
3	F3	211 ± 1.36	0.50 ± 0.08
4	F4	190 ± 2.36	0.52 ± 0.17
5	F5	217 ± 1.21	0.44 ± 0.11
6	F6	194 ± 2.16	0.51 ± 0.12
7	F7	148 ± 3.45	0.50 ± 0.04
8	F8	128 ± 2.53	0.43 ± 0.12

Table 6: % Cumulative Drug Dissolution of Pure Drug and NCs (F1 – F8)

Time (min)	%Cumulative drug dissolved								
	Pure drug	F1	F2	F3	F4	F5	F6	F7	F8
10	15.56 ± 0.36	35.83 ± 0.55	36.19 ± 0.89	40.63 ± 0.61	52.85 ± 0.89	39.72 ± 0.37	50.32 ± 0.19	53.82 ± 0.27	54.23 ± 0.69
20	20.41 ± 0.36	44.71 ± 0.34	47.25 ± 0.76	50.28 ± 0.29	61.32 ± 0.55	48.12 ± 0.28	59.65 ± 0.37	60.14 ± 0.21	63.56 ± 0.48
30	26.74 ± 0.32	54.86 ± 0.28	55.34 ± 0.86	59.49 ± 0.59	70.35 ± 0.41	56.25 ± 0.55	68.27 ± 0.87	69.52 ± 0.54	72.72 ± 0.55
40	31.93 ± 0.34	68.81 ± 0.68	70.67 ± 0.42	72.56 ± 0.64	85.31 ± 0.85	71.91 ± 0.52	82.48 ± 0.29	84.27 ± 0.72	86.84 ± 0.29
50	38.72 ± 0.37	72.14 ± 0.29	74.27 ± 0.14	76.46 ± 0.32	90.42 ± 0.23	75.27 ± 0.36	88.32 ± 0.55	90.65 ± 0.19	92.23 ± 0.38
60	44.7 ± 0.67	77.42 ± 0.36	79.21 ± 0.81	80.94 ± 0.75	95.52 ± 0.36	79.10 ± 0.57	93.85 ± 0.54	96.52 ± 0.54	98.53 ± 0.43

(mean ± SD of 3 determinations)

Table 7: Zero Order Release Kinetics Data of Pure Drug and F8 NCs

Time (min)	Cumulative % drug release	
	Pure Drug	F8 NCs
0	0	0
10	15.56	54.23
20	20.41	63.56
30	26.74	72.72
40	31.93	86.84
50	38.72	92.23
60	44.7	98.53

Table 8: First-Order Release Kinetics Data of Pure Drug and F8 NCs

Time (min)	Log Cumulative % drug remaining dissolved	
	Pure drug	F8 NCs
0	2	2
10	1.926	1.66
20	1.9	1.561

30	1.864	1.435
40	1.832	1.119
50	1.787	0.89
60	1.742	0.167

Table 9: Drug Release Kinetics of Pure Drug and F8 NCs

Formulation	Zero Order		First Order	
	Equation	R^2	Equation	R^2
Pure Drug (Atorvastatin Calcium)	y = 0.6855x + 4.8721	0.9666	y = -0.004x + 1.9844	0.9852
F8 NCs (Nanocrystals)	y = 1.4103x + 24.565	0.8286	y = -0.0267x + 2.0633	0.9168

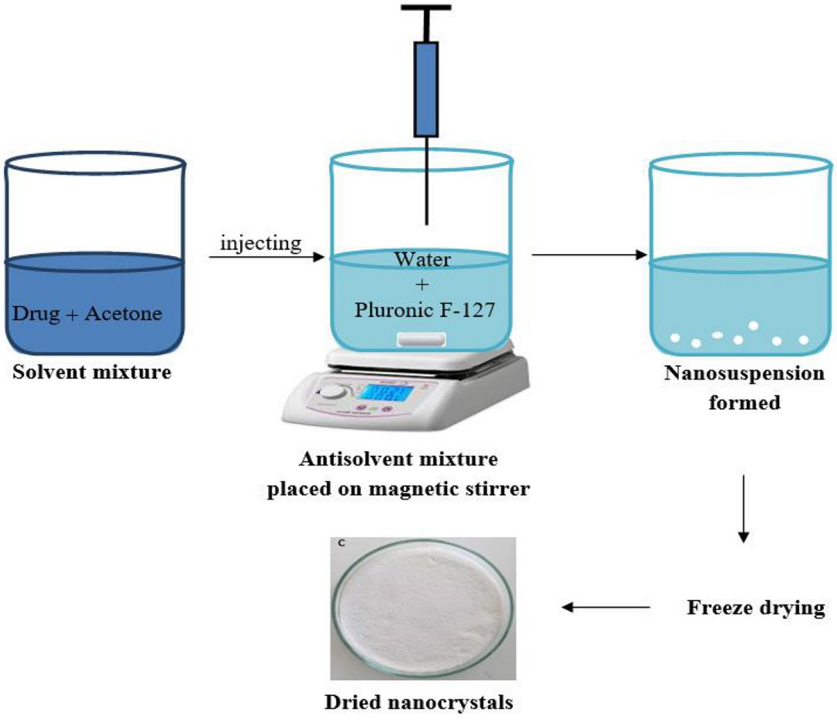

Figure 1: Schematic Representation of Solvent Antisolvent Precipitation Technique

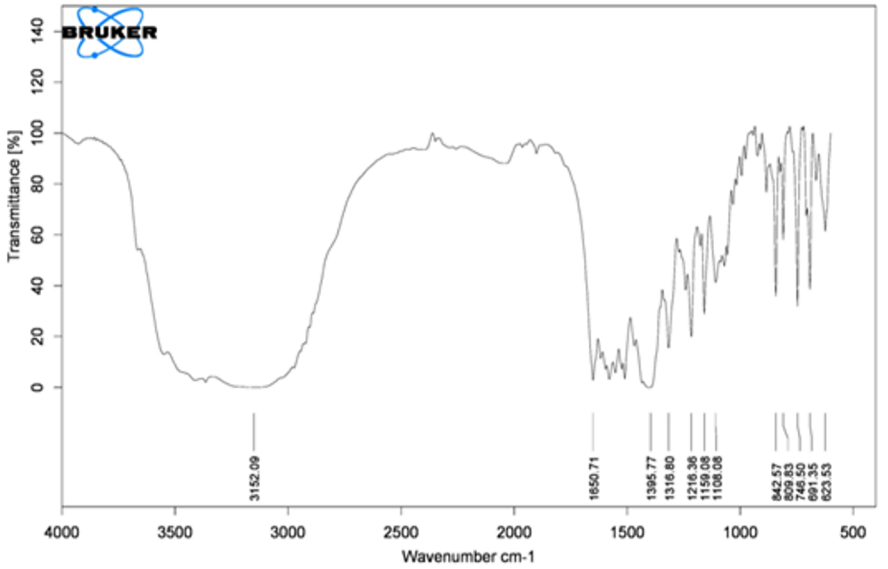

Figure 2: FT-IR Spectra of Pure ASC

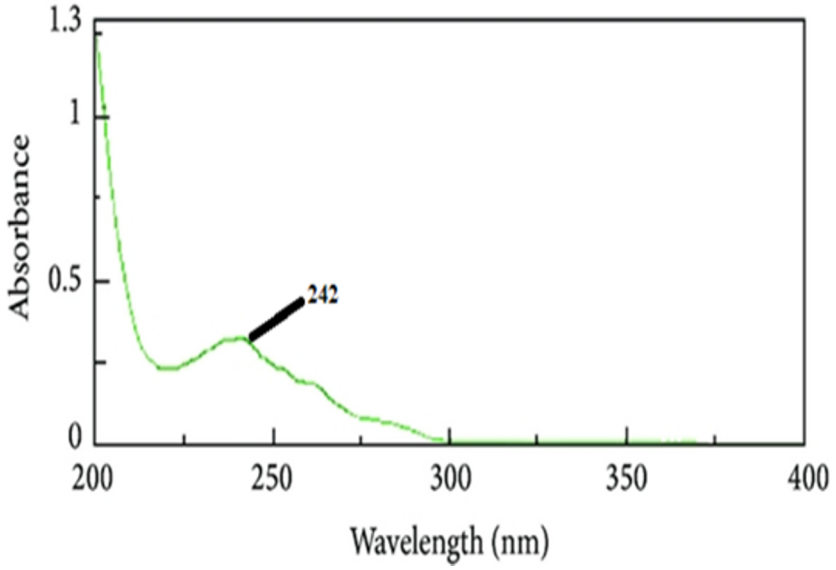

Figure 3: UV Spectra of ASC

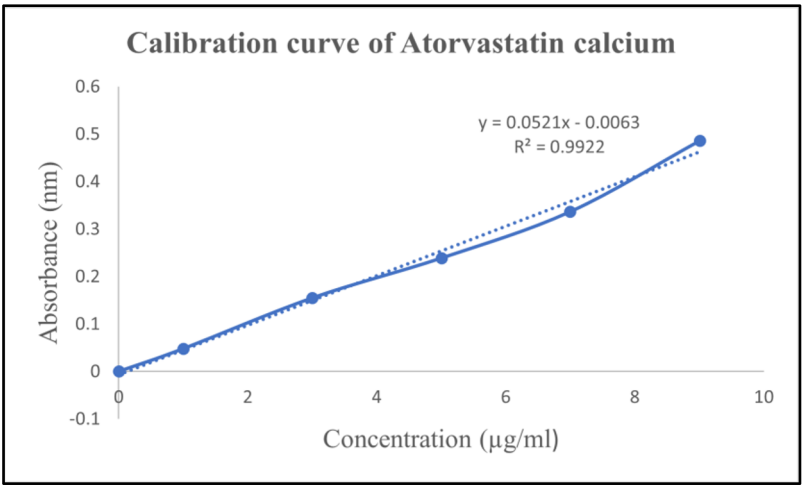

Figure 4: Calibration Curve of Atorvastatin Calcium

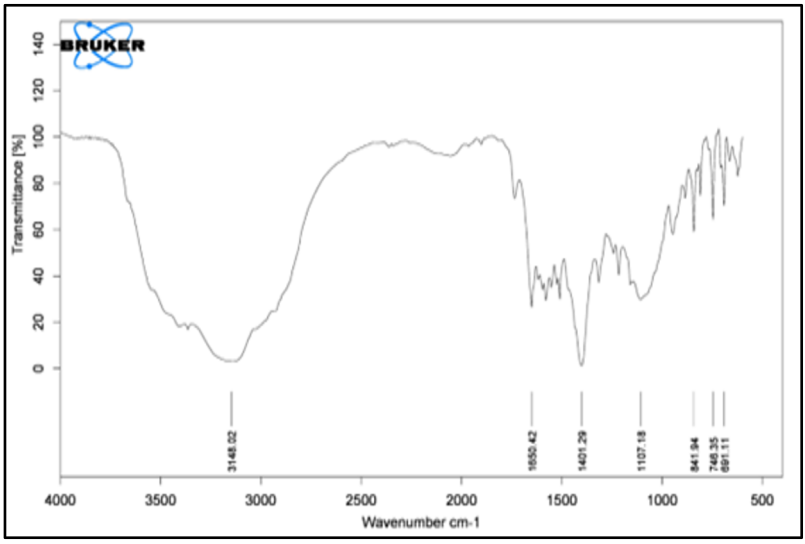

Figure 5: FT-IR spectra for Atorvastatin calcium + Pluronic F-127

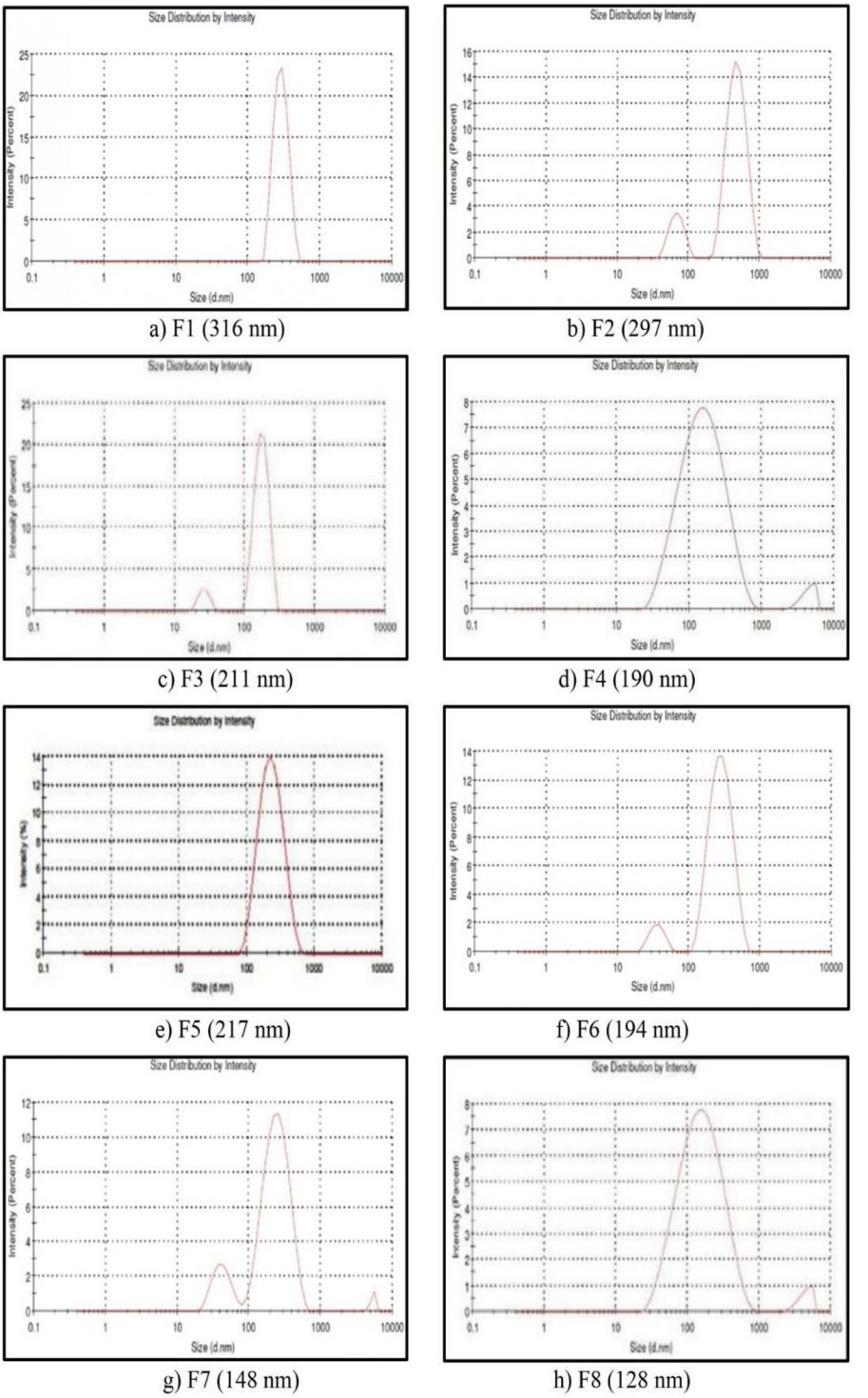

Figure 6: Particle Size Analysis of F1-F8 Formulations (a-h)

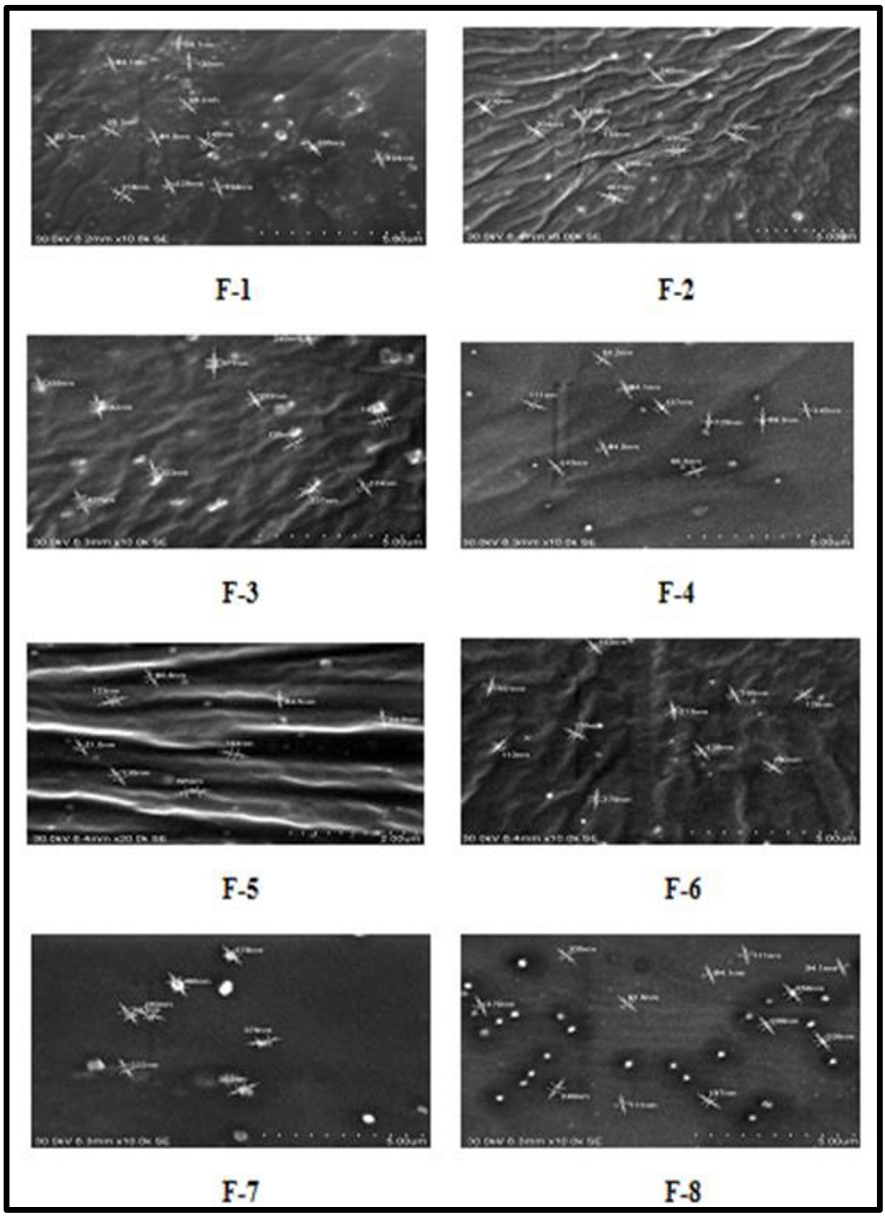

Figure 7 : Scanning Electron Micrographs of NCs of F1 – F8

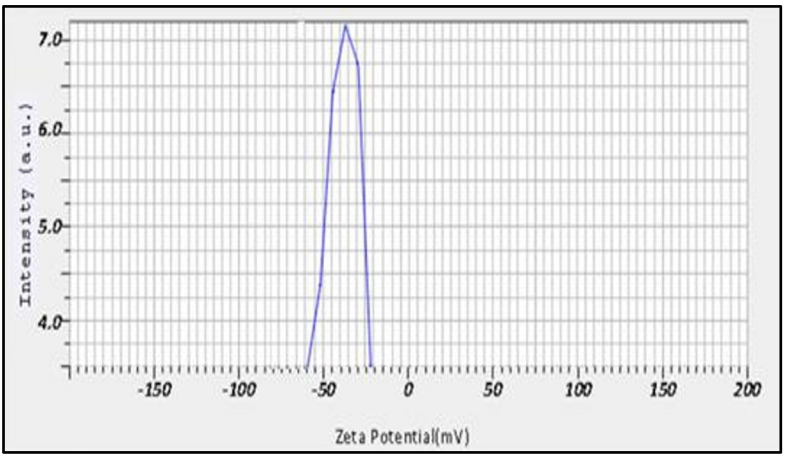

Figure 8: Zeta Potential of Ncs Formulation F8

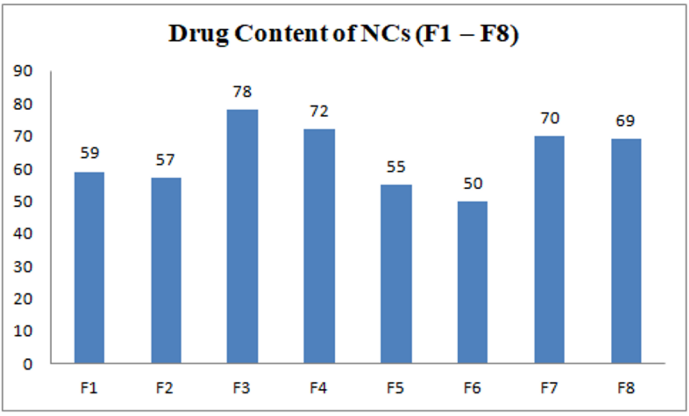

Figure 9: Drug Content (%) of NCs (F1 – F8)

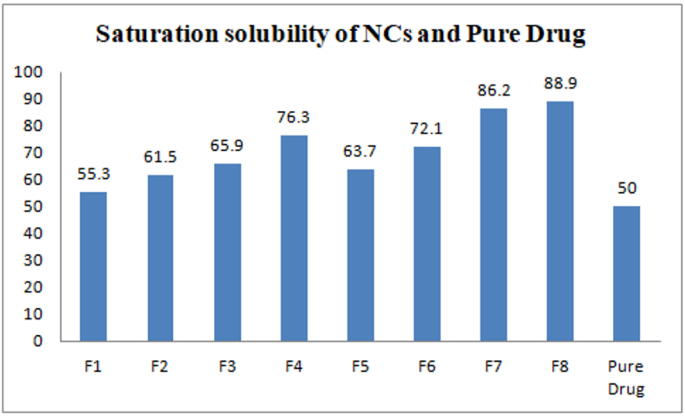

Figure 10: Saturation Solubility of NCs & Pure Drug

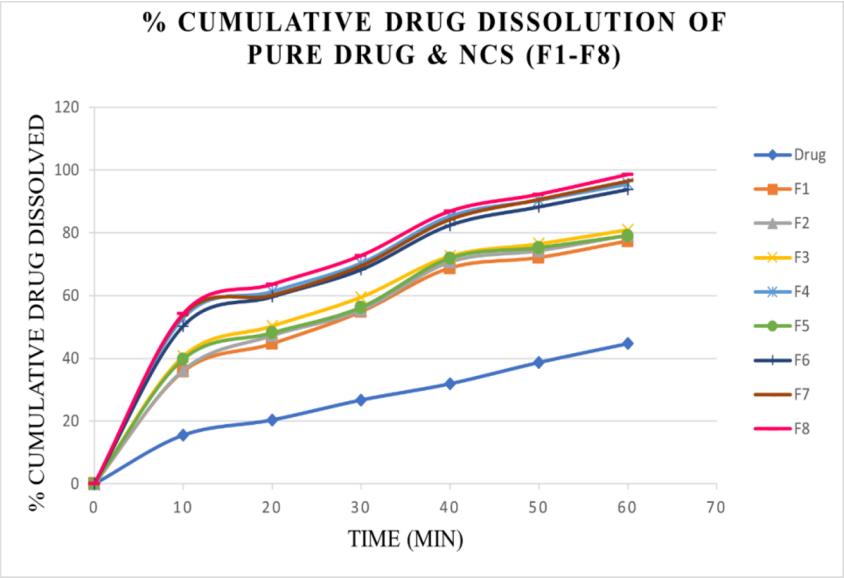

Figure 11: % Cumulative Drug Dissolution of Pure Drug and NCs (F1 – F8)

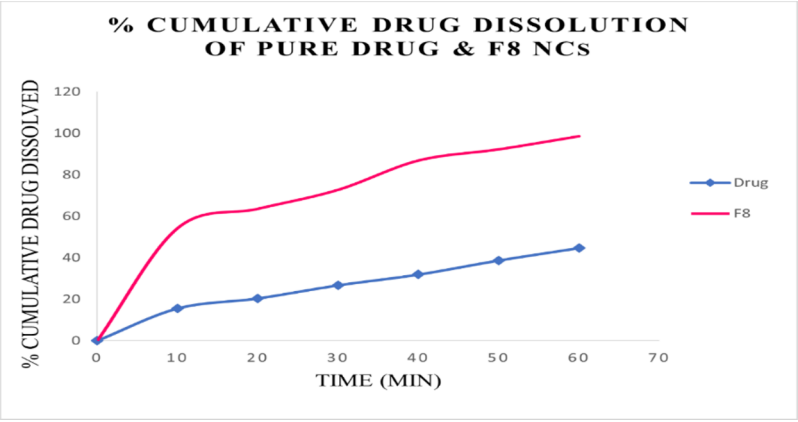

Figure 12: % Cumulative Drug Dissolution of Pure Drug and F8 NCs

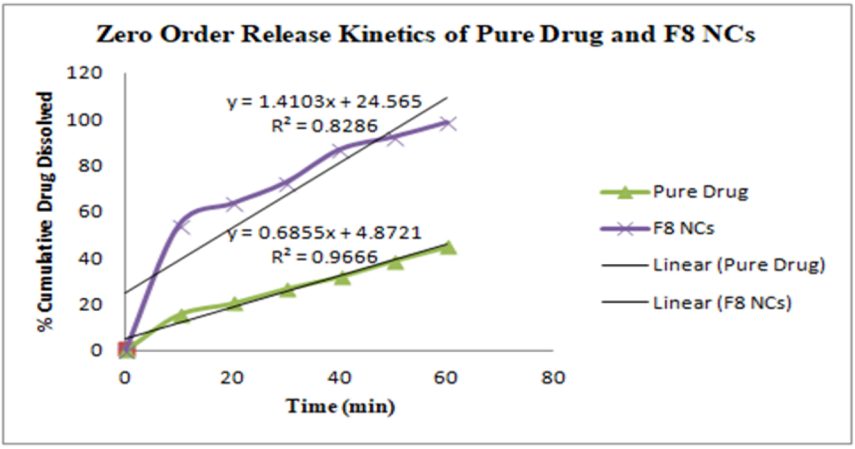

Figure 13: Zero Order Release Kinetics of Pure Drug and F8 NCs

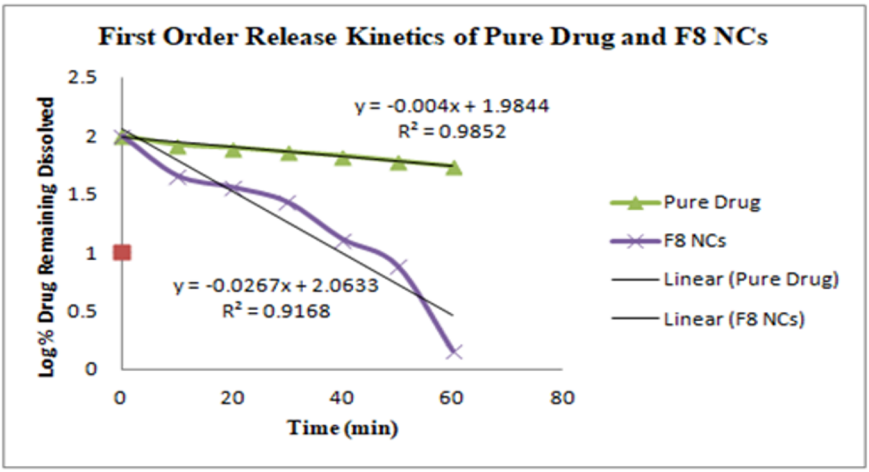

Figure 14: First Order Release Kinetics of Pure Drug and F8 NCs

4. Conclusion:

The possibility of the solvent antisolvent precipitation approach for the creation of ASC NCs was examined in the current study. The NCs were created utilizing a 2^3 factorial design and were found to have appropriate particle sizes. When compared to the pure drug, the optimized formulation demonstrated a significant rise in saturation solubility and a rapid rate of dissolution. This suggests that the development of NCs using the solvent antisolvent precipitation technique could improve the bioavailability of atorvastatin calcium and potentially solve issues with traditional formulations like tablets.

5. Acknowledgement:

We are grateful to our parents and the principal, management of VIPW for their valuable guidance throughout the completion of this work.

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