

## RESEARCH ARTICLE

# Formulation and Evaluation of Fluvastatin-loaded Nanoparticles by Nanoprecipitation Method

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## ABSTRACT

The formulation of only moderately water-soluble pharmaceuticals can benefit from the incorporation of nanoparticles, which increases the drugs' bioavailability. The primary objective of this work was to create and evaluate fluvastatin-loaded nanoparticles using the precipitation process to improve their solubility and bioavailability. Precipitation was used to prepare fluvastatin nanoparticles, which are classified as a BCS class II drug. These particles were then characterized using techniques such as Fourier transform infrared spectroscopy, differential scanning calorimetry, powder X-ray diffraction, scanning electron microscopy, zeta potential, and *in-vitro* drug release studies. There was no evidence of contact between the drug and the polymers based on the differential scanning calorimetry results, powder X-ray diffractometry, and Fourier transforms infrared spectroscopy. Images obtained by scanning electron microscopy revealed that the nanoparticles had a spherical form. The fact that the water solubility of drug-loaded nanoparticles was increased in comparison to the pure drug and that they displayed an improved dissolution profile was evidence that nanoprecipitation was a straightforward and accurate process. Both this technology on a laboratory scale and this strategy could be used to improve the solubility and bioavailability of BCS class II medications.

**Keywords:** Antilipidemic, BCS class II drug, Bioavailability, Dissolution, Fluvastatin, Nano precipitation

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## INTRODUCTION

Liposomes are the predominant type of nanoparticulate drug delivery system. Polymeric nanoparticles have particular advantages for site-specific drug delivery and for enhancing the dissolution rate along with the bioavailability of medicines that are only partially water-soluble.<sup>1</sup> The generation of drug-loaded nanoparticles is a strategy that possesses a great deal of potential. It is possible to accomplish particle size reduction into the nanometer range utilizing a variety of ways, and each of these techniques has been described in some detail.<sup>2</sup> Insufficient bioavailability is often the result of drugs in the Biopharmaceutical Classification System (BCS) class II having poor solubility and a low dissolution rate in the aqueous gastrointestinal fluids. This insufficient bioavailability can only be improved by increasing the solubility and dissolution

rate of the drug through the use of a variety of innovative techniques.<sup>3</sup> Solid dispersion and the production of inclusion complexes, microparticles, and nanoparticles are some of the methods that can be used to increase the pace at which drugs dissolve in the body.

Nanoparticles are colloidal particles that range in size from 10 to 1000 nm and are characterised by the fact that the active ingredients (medication or other biologically active material) are dissolved or entrapped inside them.<sup>4</sup> Nanospheres, nanocapsules, dendrimers, solid-lipid nanoparticles, polymeric micelles, and liposomes are only a few of the different varieties that fall under this category. Because of advancements in nanotechnology, it is now possible to manufacture drug nanoparticles that can be applied in a wide variety of creative new ways to treat several illnesses. There are currently new

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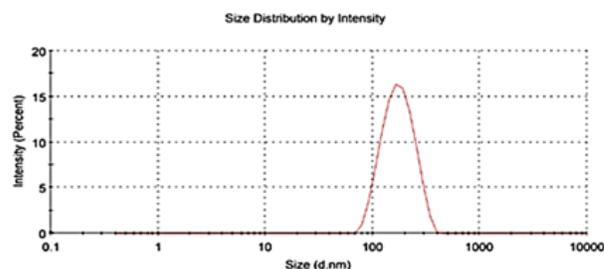
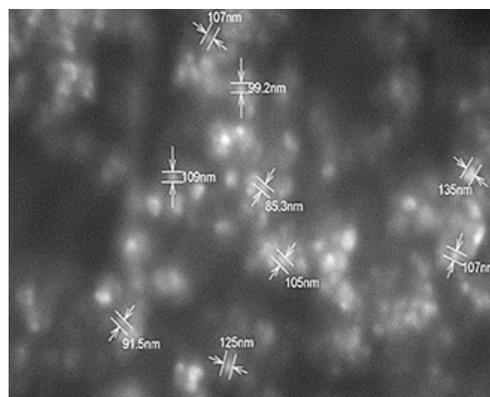
**Table 1:** Formulation design for FLNs

Ingredients	F1	F2	F3	F4	F5
Fluvastatin (mg)	40	40	40	40	40
Chitosan (mg)	0.25	0.5	0.75	1	1.25
Acetic Acid (%V/V)	2	2	2	2	2
Sodium Tripolyphosphate	1	1	1	1	1

medication delivery methods that can be employed to improve the efficacy of drugs while also reducing their negative effects.<sup>5</sup> Nanoparticles composed of solid lipids are a part of the fast-emerging field of nanotechnology. They have many possible applications in clinical care as well as in research. When it comes to the delivery of medicinal medications, nanoparticles are garnering a significant amount of interest. It is now conceivable, depending on the physicochemical properties of a drug, to select the optimal method of production along with the optimal polymer to accomplish effective entrapment of the drug.<sup>6</sup> This is made possible by the fact that it is now feasible to do so. There are a variety of techniques that can be utilised in the process of preparing nanoparticles. Some of these techniques include solvent evaporation, nanoprecipitation, emulsification/solvent diffusion, salting out, dialysis, supercritical fluid technology, and rapid expansion of supercritical solution into the liquid solvent.

The nanoprecipitation technique, also known as the solvent displacement method, is a procedure that is uncomplicated, quick, and simple to carry out. It requires the formation of a precipitate of a premade polymer from an organic solution, followed by the diffusion of an organic solvent in an aqueous medium, with or without the participation of a surfactant. It requires two solvents that can be dissolved in water without causing any problems. In a perfect world, the polymer and the medicine would both dissolve in one of the solvents but remain insoluble in the other (non-solvent). When the polymer solution is mixed with the non-solvent, a nanoprecipitation process takes place that is characterised by a fast desolvation of the polymer (aqueous solution). The polymer precipitates as soon as the organic solvent carrying the polymer has been completely displaced by the aqueous medium, which results in the instantaneous entrapping of the drug. The instantaneous production of a colloidal suspension is brought about by the deposition of the polymer at the interface between the organic solvent and the water, which is brought about by the rapid diffusion of the organic solvent. This method works best with hydrophobic chemicals that are soluble in ethanol or acetone but have a very low level of solubility in water.

Fluvastatin is a type of medication known as an antilipidemic drug. It works by lowering levels of cholesterol as well as triglycerides in the blood. In the treatment of hypercholesterolemia and hypertriglyceridemia, it can be administered on its own or in combination with statins.<sup>7</sup> The study that is being presented here focuses on the synthesis and analysis of fluvastatin nanoparticles (Table 1) that have been loaded using the precipitation method.

**Figure 1:** Size distribution of F5**Figure 2:** SEM image of formulation F5

## MATERIALS AND METHODS

A free trial supply of fluvastatin was generously provided by Pharmatrian in Hyderabad. Narmada Chemicals was the supplier for the chitosan and sodium tripolyphosphate that was acquired. The analytical grade was employed for all other components, materials, or substances.

### Method of Preparation of FNPs

The synthesis of nanoparticles by a process known as nanoprecipitation. In this technique, the polymer is dissolved by using an acetic acid solution that has been previously prepared. The medication and its constituents were dissolved in the appropriate solvent. After adding the medication solution to the polymer solution, the resulting mixture turned out to be a viscous solution. The solution was then stirred for two hours using a magnetic stirrer at a rotating speed of between 500 and 700 revolutions per minute. After that, we continued to sonicate that solution for another hours. The precipitation that had settled was spun in a centrifuge for five minutes. The precipitation that was produced is collected and put into an oven with heated air for 1-hour to produce nanoparticles.

### Evaluation of FLNs

#### Particle Size and Zeta Potential

The method of dynamic laser scattering was utilised to do the particle size analysis. The particle size and PDI of NPs were determined using a Zetasizer (Nano ZS, manufactured by Malvern Instruments in Malvern, UK). To get a better idea of the size of the particles, the NPS suspension was watered down

**Table 2:** Evaluation of FNPs

Formulation code	Particle size	Zeta potential	Practical yield (%)	Entrapment efficiency (%)	Drug loading (%)
F1	225 ± 0.25	0.92 ± 0.84	61.25 ± 0.84	59.46 ± 0.91	48.75
F2	194 ± 0.34	0.88 ± 0.12	66.67 ± 0.63	62.67 ± 1.22	47.3
F3	172 ± 0.66	0.76 ± 0.24	79.37 ± 0.79	77.12 ± 1.39	72.8
F4	168 ± 0.28	0.64 ± 0.38	92.38 ± 1.04	91.48 ± 1.26	67.1
F5	123 ± 0.54	0.52 ± 0.57	94.41 ± 0.64	94.11 ± 1.4	68.75

Mean ± SD, n=3

**Table 3:** FT-IR interpretation of pure drug and optimized formulation

S. no.	Functional group	Observed wave number (cm <sup>-1</sup> )	
		Pure API	Optimized formulation
1	C-H bending	740.0	755.96
2	C = C strength	1479.3	1502.8
3	C = O strength	1799.53	1819.25
4	C – H strength	3020.86	3122.45
5	O – H strength	3318.84	3489.48
6	N – H Strength	3444.0	3448.0

with distilled water. After taking twelve separate measurements, an average was calculated for the results.

#### Entrapment Efficiency (EE)

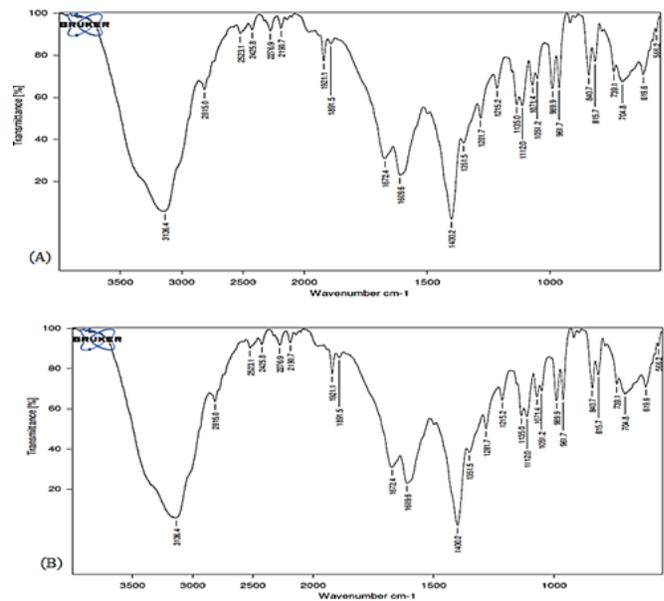
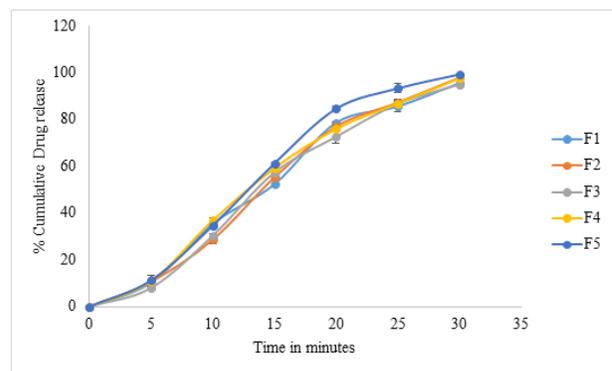
An indirect method was utilised to determine the entrapment efficiency (EE) of the nanoparticles found in fluvastatin. In a nutshell, the suspension containing the NPs had been centrifuged for ten minutes at a speed of twelve thousand revolutions per minute. The recovered supernatant was diluted with methanol, and the volume of free fluvastatin was measured using a spectrophotometer set at 238 nm wavelength. The amount of UV-visible light that was used was measured in the supernatant.

The EE was calculated using the formulation as follows:

$$\% \text{ Entrapment Efficiency} = \left( \frac{\text{Amount of Fluvastatin added in formulation} - \text{Amount present in the supernatant}}{\text{Amount of fluvastatin added in formulation}} \right) \times 100$$

#### The Percentage Yield of Nanoparticles

The yield of nanoparticles was determined by comparing the whole nanoparticle formed against the combined weight of the copolymer and drug

$$\% \text{ Yield} = \left( \frac{\text{Amount of Drug}}{\text{Amount of drug} + \text{polymer}} \right) \times 100$$

**Figure 3:** FT-IR spectra of (A) pure drug (B) Optimized formulation

**Figure 4:** %Cumulative drug release of formulations F1 to F5.

**Table 4:** %Cumulative drug release of formulations F1 to F5.

Time (Mins)	F1	F2	F3	F4	F5
0	0	0	0	0	0
5	10.24 ± 1.24	10.65 ± 0.28	7.96 ± 0.68	10.86 ± 2.34	11.2 ± 1.95
10	35.42 ± 0.24	28.63 ± 1.65	30.32 ± 1.08	35.42 ± 1.32	34.65 ± 1.25
15	54.35 ± 1.42	62.45 ± 0.68	54.54 ± 1.57	54.68 ± 1.32	54.69 ± 0.24
20	78.65 ± 0.24	84.65 ± 1.24	72.65 ± 2.65	75.98 ± 0.38	76.98 ± 0.98
25	85.65 ± 2.34	93.35 ± 1.01	86.79 ± 0.68	86.94 ± 0.86	87.32 ± 1.86
30	95.64 ± 0.36	97.95 ± 0.68	91.01 ± 0.12	97.64 ± 0.32	99.36 ± 0.28

### Drug Load

After determining their mass, nanoparticles containing fluvastatin were dissolved in fifty millilitres of methanol. To completely dissolve the GTE into the methanol, it was sonicated for 15 minutes. Following filtration of the solution onto Whatman filter paper, the resultant filtrate was further processed by the addition of methanol. It was determined the aliquot that was scanned at a wavelength of 296 nm using UV-visible spectroscopy and the amount of medication that was present.

### *In-vitro* Drug Release Characteristics

The *in-vitro* drug diffusion from the formulation was investigated using USP-II devices. As a medium dissolving solution, the buffered phosphate solution with a pH of 6.8 was used. Utilize the nanoparticles that have been created in a variety of compositions. It is necessary to replace the 5 mL sample with a buffer solution at regular intervals of 5 minutes, such as 5, 10, 15, etc. Using a UV spectrometer, conduct an analysis of the material at 238 nm.

### Morphological Studies by Scanning- Electron Microscopy

Scanning electron microscopy was utilised to investigate the morphology of nanoparticles. Before performing the SEM examination, a preliminary step consisted of placing 100 microliters (l) of the formulation of chitosan nanoparticles into a glass slide measuring 10 millimetres (mm) and allowing it to dry in a vacuum desiccator at room temperature for one full day. To prepare nanoparticles for examination in a higher vacuum evaporator, sufficient supports were attached to them, and then a gold sputter module was used to coat them with gold. The examination was carried out utilising a specialised magnification at 15 kv.

### Drug-Excipient Compatibility Studies

The FT-IR spectrophotometer was utilised throughout the drug excipient compatibility tests that were carried out (Perkin Elmer). Separate FT-IR analyses of the medication, polymers, and formulations were performed, and the results were correlated to determine whether or not they were compatible.

## RESULTS AND DISCUSSION

It was determined through the use of the human eye that the fluvastatin sample was in the form of a yellow powder. The reported melting point of fluvastatin was discovered to be 217 degrees, which served as confirmation that the medicine was pure. It was discovered that the nanoparticles are a powder that is amorphous, white, and odorless.

Table 2 presents the nanoparticles' average particle size distributions for your perusal. It was discovered that the average particle size of nanoparticles ranged from 225.025 to 123.054 nm. According to one definition<sup>16</sup> the zeta potential is the electrical potential between the medium and the fluid layer associated with the dispersed particles. The zeta potential is one of the fundamental characteristics that is known to determine a substance's level of stability. It is a measure of the magnitude

of the electrostatic or charges repulsion or attraction that exists between particles. It was discovered that the zeta potential of the produced nanoparticles fell somewhere in the range of 0.92 to 0.52 mV. It was discovered that increasing the zeta potential led to a decrease in the amount of particle aggregation that occurred as a result of electric repulsion, which increased the stability of nanoparticles. The value of the zeta potential for batch F5 was calculated to be 0.52 mV. It has been found that the presence of stable groups of chitosan on the surface of nanoparticles can be ascribed to the appearance of a positive charge on the surface of the nanoparticles. Figure 1 illustrates how small nanoparticles are by showing their dimensions.

Table 2 contains information regarding the effective yield, drug loading, and EE. The actual yield of the produced nanoparticles was anywhere between 61.25 and 84.1%, and it was as high as 94.41%. The drug concentration ratio to polymer amount increased the number of nanoparticles produced. It was discovered that the real drug loading and EE increased along with the amount of polymer that was used in the formulation. It was determined that the EE fell somewhere in the range of 59.46 to 94.11%. It was discovered that the percentage of medication loaded into nanoparticles fell somewhere in the region of 48.75 to 68.75%. It was found that the concentration of the polymer, the ratio of the solvent, and the stirring rate all affect the amount of medicine encapsulated and the efficiency of the process. It was determined that batch F5 was the most effective for manufacturing nanoparticles because it had a high yield, contained the real amount of medication, and encapsulated it effectively.

### Morphological Studies by SEM

SEM photograph was shown in Figure 2, SEM has shown that the nanoparticles were small, spherical and porous.

### FT-IR Study

It was abundantly obvious from the FT-IR data that the functions of the medicine, particularly the intensities of the peak, had not been altered in any way. This leads one to believe that the polymer has not reacted with the medicine at any point during the formulation process to produce any reactive products. Therefore, it is merely a physical mixture, and there is no interaction between them, which is good news since it means that the formulation process may move forward. Table 3 and Figures 3(A) and 3(B) display the FT-IR spectra of the drug and the drug combined with excipients, respectively (B).

### *In-vitro* Drug Release Studies

The dialysis bag was utilized throughout the *in-vitro* medication release testing that was carried out. The information on the cumulative percentage of drug release from the formulations was shown in Table 4 and Figure 4. For thirty minutes, the *in vitro* drug release profile of the formulation fell somewhere in the range of 91.01 to 99.36%. The disintegration rate for batch F5 was the quickest overall, with roughly 99.36% of the medication being released within the first thirty minutes.

**CONCLUSION**

Formulation F5, which contained chitosan in a ratio of 1:1.25 drug to polymer, demonstrated good results among the several nanoparticulate formulations that were created using the nanoprecipitation process. The FT-IR investigation concluded that there was no significant interaction between the medication and the polymers that were used in this study. Therefore, the approach used to address the poor solubility and bioavailability of the medicine fluvastatin nanoparticles was successful.

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**CONFLICTS OF INTEREST**

There are no conflicts of interest.

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