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Original Article

Design and characterization of nanoparticles containing ezetimibe and atorvastatin calcium

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ABSTRACT

Ezetimibe is a weak hypolipidemic drug with poor aqueous solubility; with bioavailability of only 12% therefore large dose is prescribed which leads to adverse effects on continuous usage. Combination of Ezetimibe and low dose of statins is as effective in lowering LDL-CH. Therefore Atorvastatin is used with Ezetimibe in this study. In the present study Atorvastatin calcium and Ezetimibe nanoparticles were prepared and characterized for particle size analysis, drug entrapment efficiency, scanning electron microscopy, Zeta potential and *in vitro* studies. All prepared formulations (F1 to F4) resulted in the nanosize range of 59.20 ± 11.75 nm to 92.10 ± 13.58 nm with spherical morphology. Drug entrapment efficiency and percentage yield were 37.01±0.12% to 62.00±0.29% and 43.88±0.77% to 64.41±0.55 % for Atorvastatin and Ezetimibe respectively. Nanoparticles of optimized formulation (F3) showed Zeta potential of 15.3 mV. *In vitro* release profile of nanoparticulate formulations F1 to F4 showed 79.95 to 84.60% of Atorvastatin Calcium and 79.09 to 85.09% of Ezetimibe drug release over a period of 240 min. The results indicated that formulation (F3) could be utilized as potential delivery system for the treatment of cardiovascular disease and an approach to reduce undue adverse effects of the drug due to over dosage.

Keywords: Atorvastatin calcium, Ezetimibe, Eudragit RLPO, Nanoprecipitation

INTRODUCTION

The field of nanotechnology is emerging worldwide with tremendous investment in it. Nanotechnology is defined as the science and engineering involving design, synthesis, characterization of materials whose smallest functional organisation is one billionth of a meter (10^{-9}). These nano sized objects especially the nanoparticles has the ability to improve pharmacokinetics & increased bio distribution of therapeutic agents to target organ. With the development of nanoparticles the required dose is lowered with increased efficacy as well as increased therapeutic indices & safety profile of drug.[1]

Polymeric nanoparticles prepared from natural polymers have received a majority of attention due to their solubility and ease of surface modification. They can be made to achieve controlled drug release to disease specific localisation by turning the polymer characteristics and surface chemistry.[2]

An advantage of nanoparticles for drug delivery arises from its basic properties *i.e.*, small size and use of biodegradable materials. Because of their small size Nanoparticles can extravagate through the endothelium, epithelium or penetrate through capillaries. Nanosize of these particles allows for efficient uptake by a variety of cell types and selective drug accumulation at target sites. They offer another advantage over larger microparticles because they are better suited for IV delivery.

Through this research an attempt is made to design nanoparticles for prevention of atherosclerosis formation using synthetic polymer as a drug carrier. Atherosclerosis is a vascular disease characterised by thickening of artery wall as a result of accumulation of calcium & fatty materials such as cholesterol and TGs. It reduces the elasticity of the artery wall and therefore less blood travel through it, resulting in hypertension, hypoxia and cardiac arrest. Hypolipidemic drugs mainly statins are ideal to reduce fatty acid levels in the body which prevents atherosclerosis formation.[3, 4]

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Hypolipidemic drugs used in this research are Ezetimibe and Atorvastatin Calcium. Ezetimibe is a hypolipidemic drug with t½ of 22 hrs. Due to its poor aqueous solubility it is not well absorbed hence its bioavailability is less. It is a potent cholesterol absorption inhibitor and has been shown to significantly lower serum cholesterol concentrations by selectively inhibiting cholesterol uptake at the brush border of the small intestine. Ezetimibe effectively inhibits the intestinal absorption of cholesterol and plant sterols without affecting absorption of triglycerides, fatty acids, bile acids or fat-soluble vitamins. It is mainly used as a supplement to statins. Combination of Ezetimibe and low dose of statins is as effective in lowering LDL-CH as high dose of statins alone. Therefore Atorvastatin is used in combination with Ezetimibe in the present study.[5]

Atorvastatin has highest LDL-CH lowering efficacy at minimal dose of 10 mg. It has t½ of 14 hrs and has antioxidant property as well. The BCS system classified Atorvastatin as class II drug having low aqueous solubility & variable absorption. It is insoluble in aqueous solution at pH4 and below but slightly soluble in water. The intestinal permeability of Atorvastatin is high at physiological relevant intestinal pH. However, it has been reported that the absolute bioavailability of Atorvastatin is only 12% after a 40 mg of oral dose. The low systemic availability attributed to low dissolution, pre-systemic clearance in gastrointestinal mucosa and hepatic first pass metabolism.[6]

The poor bioavailability necessitates development of efficient system which can deliver drug orally with increasing bioavailability of Atorvastatin. Therefore Atorvastatin in combination with Ezetimibe is formulated into nanoparticles.

This research aims at formulation and development of optimised nanoparticles of hypolipidemic drugs *viz*., Atorvastatin Calcium and Ezetimibe as combination therapy for cardiovascular disease. It also aims at characterisation of the formulated nanoparticles for entrapment efficiency, particle size, morphology, etc.

Objectives of the Study

- To perform preformulation studies of the drug and other excipients.
- To prepare Nanoparticles of Atorvastatin Calcium and Ezetimibe for treatment of cardiovascular disease.
- To evaluate the prepared nanoparticles for particle size, % EE, % yield, Zetapotential and *in-vitro* drug release studies.

MATERIALS AND METHODS

Materials: Atorvastatin Calcium and Ezetimibe were received as a gift sample from IPCA lab, Mumbai and MSN lab, Hyderabad respectively. Eudragit RLPO and Pluronic F68 were obtained as gift sample from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All other chemicals and solvents were of analytical reagent grade and were used without further purification.

Compatibility studies: Compatibility of the drug Atorvastatin calcium and Ezetimibe with Eudragit RLPO, used to formulate nanoparticles was established by IR spectroscopy method. FT-IR spectral measurement for pure Atorvastatin calcium, Ezetimibe and Eudragit RLPO and physical mixtures of Atorvastatin calcium, Ezetimibe and Eudragit RLPO were taken at ambient temperature and studied over a frequency range of 4000-650 cm^{-1}.

Preparation of Atorvastatin calcium and Ezetimibe Nanoparticles[7]: Atorvastatin calcium and Ezetimibe with Eudragit RLPO nanoparticles were prepared by nanoprecipitation method. Four different formulations F1, F2, F3, and F4 were prepared by changing the polymer ratio as 1:1, 1:2, 1:3 and 1:4 respectively.

Drug and polymer was first dissolved in Acetone (15 ml) (organic phase). This organic phase was injected at the rate of 5ml/min in 20 ml of water containing Pluronic F68 (10mg) under stirring at room temperature. Acetone was evaporated under reduced pressure. After that aqueous colloidal mixture was centrifuged and lyophilized to obtain dry powder of nanoparticles. Compositions of formulations are shown in Table no I.

EVALUATION OF NANOPARTICLES[7]

Particle size analysis: The size distributions along the volume mean diameter of the nanoparticle was measured by Dynamic Light Scattering technique using Nanotrac Particle Size Analyzer (Microtrac, CA, USA).

Polydispersity index: The polydispersity index (PDI) was also measured by Dynamic light scattering Instrument. PDI is calculated using the following equation (1).

$PDI = \Delta d/d_{avg}$ …… Eq. (1)

Where, Δd is the width of distribution denoted as SD and d_{avg} is the average particle size denoted as MV (nm) in particle size data sheet.

Zeta potential: Nanoparticles were characterized with respect to zeta potential (NPA152-31A Zetatrac, Microtrac, USA) by using dynamic light

scattering technology joined with the interaction of random Brownian motion with driven electric field motion of particle suspensions.

Shape and surface Morphology: Particle morphology of the Atorvatstain Calcium and Ezetimibe nanoparticles is performed by Scanning Electron Microscopy (SEM) to determine the surface morphology, size and shape of formulation and to observe the aggregation property of nanoparticles with carrier particles.

Percentage entrapment efficiency: The freeze-dried nanoparticles recovered were dissolved in 50 ml of methanol and then subjected to ultracentrifugation for one hour at 10,000 rpm. The amounts of drugs present in the supernatant were analyzed using an ultraviolet-visible spectrophotometer at the highest spectrum wavelengths of 246 nm and 233 nm. A standard calibration curve was constructed, using different concentrations of Atorvastatin calcium and Ezetimibe (5–30 μg) versus maximum absorbance. The maximum absorbance of the supernatants of drug-loaded nanoparticles was used for quantification of Atorvastatin calcium and Ezetimibe at wavelengths of 246 nm and 233 nm, respectively.

Percentage yield: The nanoparticles from each formulation were weighed and the respective percentage yield was calculated.

***In Vitro* drug release[8, 9]:** Atorvastatin calcium - Ezetimibe with Eudragit RLPO nanoparticles (20 mg) were dispersed in 5 ml of phosphate buffer (pH 6.8) and then put in the dialysis bags with a molecular weight cut-off of 12,000–14,000 Da. The bags were hermetically sealed and immersed in a beaker containing 100 ml of phosphate buffer of pH 6.8. The contents were stirred continuously with a magnetic stirrer at 50 rpm with temperature adjusted to 37ºC. At predetermined time intervals, 1 ml of dispersion was removed and analyzed for drug content by UV-spectrophotometry at 246 and 233 nm.

RESULTS & DISCUSSION

Pre-formulation studies

FTIR study: The FTIR spectrum of the pure Atorvastatin calcium and Ezetimibe and physical mixture of API and Eudragit RLPO were recorded by FTIR spectrometer which was compared with standard functional group frequencies of Atorvastatin calcium and Ezetimibe. The functional group frequencies of Atorvastatin calcium and Ezetimibe were in the reported range which indicates that the obtained sample of Atorvastatin calcium and Ezetimibe is pure.

Compatibility studies

FTIR study: Compatibility studies of pure drugs with Eudragit RLPO were carried out. IR spectra of pure drugs and combination of drugs and Eudragit RLPO were obtained and are shown in Table No II. All the characteristics peaks of Atorvastatin Calcium and Ezetimibe were present in the spectra thus indicating compatibility between the drugs and polymer which confirmed that there were no significant changes in the chemical integrity of the drug.

Evaluation of Atorvastatin Calcium and Ezetimibe Nanoparticles

Particle size and Size distribution: Particle size and size distribution are very important parameters for oral delivery purpose in order to increase the bioavailability of the drug. The mean particle size for formulations F1 to F4 varied in range of 50nm to 100nm the data is shown in Table no III. In general, mean diameter of particle increased with increasing the polymer concentration.

Shape and surface morphology: Nanoparticle surface morphology and shape were visualized using SEM (JSM-T330A, JEOL) with magnification of 15000 to 75000 X for capturing the SEM images. The drug loaded nanoparticles of Formulation F3 was found to be spherical which is shown in Fig II.

Polydispersity index: Polydispersity index (PI) indicates the width of the particle size distribution, which ranges from 0 to 1. PI measurement was essential to confirm the narrow size distribution of the particles. The mean polydispersity index values for the drug loaded formulations varied in the range of 0.2752 to 0.973 (Table no III). It could be inferred that all the formulations showed mid-range polydispersity.

Zeta potential: Zeta potential is an important parameter to analyze the long-term stability of the nanoparticles. Generally higher zeta potential values, both (+) or (−) indicate long-term stability because of electrostatic repulsions between particles with same charges avoid aggregation of particles. Zeta potential for optimized formulation (F3) was 15.3 mV as shown in the Fig III which may result in good dispersion stability. The higher value of the zeta potential indicates a more stable suspension.

Entrapment efficiency: The percentage entrapment efficiency was increased from F1 to F3 and then decreased in F4. Increase in the polymer concentration causes increase in the drug entrapment until saturation of drug and polymer is attained, further increase in the polymer

concentration leads to decrease in the entrapment efficiency the results are shown in Table no III.

Percentage yield: As the concentration of polymer increases there is increase in the yield and maximum yield obtained was found to be 64.41±0.5% and 59.09±0.12% for the formulation F4 containing Atorvastatin calcium and Ezetimibe respectively, the results are as shown in Table no III.

***In vitro* drug release:** *In vitro* drug release of Atorvastatin calcium and Ezetimibe nanoparticles was dependent upon the size of the nanoparticles and concentration of polymer. *In vitro* drug release from the nanoparticles in Phosphate buffer pH 6.8 was performed using dialysis bag diffusion technique. The small particles with less polymer concentration exhibited more drug release rather than larger particles with more polymer concentration which is shown in Table no IV and V.

CONCLUSIONS

Our study demonstrates that nanoparticles of Atorvastatin Calcium and Ezetimibe can be prepared using Eudragit RLPO as polymer and Pluronic F68 as the stabilizer. The nanoparticles were spherical in shape with a size of 73.80 ±16.48nm. (Table no III). Drug entrapment efficiency was found to be highest 62.00% and 51.11% for Atorvastatin calcium and Ezetimibe in formulation F3. Furthermore, the percentage yield of nanoparticles was found to be 60.00±0.59%. The results indicated that formulations of Atorvastatin calcium and Ezetimibe loaded nanoparticles could be utilized as potential delivery system for the treatment of cardiovascular disease and formulation (F3) has great scope for development of *in vivo* studies and long term stability studies.

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Table I: Composition of Formulations F-1 to F-4

Formulation code	Atorvastatin calcium (mg)	Ezetimibe (mg)	Eudragit RLPO (ml)	Pluronic F68 (mg)	Acetone (ml)	Water (ml)
F1	5	5	5	10	15	20
F2	5	5	10	10	15	20
F3	5	5	15	10	15	20
F4	5	5	20	10	15	20

Table II: FTIR spectra of pure drug and drug-polymer mixture

Functional groups	Peaks observed (Cm^{-1}) ATORVASTATIN CALCIUM	EZETIMIBE	EUDRAGIT RLPO	MIXTURE
C-C Stretch	1656.97	1602.00	1662.88	1690.21
C-H Stretch	2920.00	2958.11	2925.01	2950.00
C-O Stretch	1065.09	1068.60	1065.12	1065.81
C=O Stretch	1785.98	1716.07	1720.00	1720.00

Table III: Particle Size, PDI, % EE and % Yield Values of Nanoparticles Formulations F1 to F4

Formulation	Drug : polymer ratio	% of EE*		% Yield		Particle size (nm)	PDI*
		ATC	EZE	ATC	EZE		
F1	1:1	37.01±0.12	33..09±0.17	45.97±0.41	43.88±0.77	59.20±11.75	0.428±0.006
F2	1:2	51.46±0.24	49.44±0.27	56.58±0.60	47.09±0.12	72.20 ±10.67	0.973±0.008
F3	1:3	**62.00±0.29**	**51.11±0.11**	**60.00±0.59**	**54.23±0.87**	**73.80 ±16.48**	**0.275±0.012**
F4	1:4	58.20±0.34	50.04±0.54	64.41±0.55	59.09±0.12	92.10 ±13.58	0.287±0.017

*Data expressed in Mean± S.D (n=3)

Table IV: *In Vitro* Drug Release Profile of Nanoparticles Formulation F1 to F4 (Atorvastatin Calcium)

TIME (MIN)	%CDR			
	F1	F2	F3	F4
30	14.61	9.70	11.49	16.67
60	22.50	12.24	19.80	21.56
90	29.78	17.87	26.87	27.60
120	41.32	27.84	32.38	35.78
150	61.45	35.97	57.63	49.70
180	74.80	59.80	67.80	57.06
210	80.60	78.38	76.50	69.78
240	84.60	81.43	80.64	79.95

Table V: *In Vitro* Drug Release Profile of Nanoparticles Formulation F1 to F4 (Ezetimibe)

TIME (MIN)	%CDR			
	F1	F2	F3	F4
30	15.09	10.19	13.09	16.67
60	23.11	15.09	20.80	20.56
90	28.70	22.13	23.78	26.87
120	40.90	29.22	33.83	36.32
150	58.76	49.99	55.36	48.45
180	69.88	61.05	66.08	57.06
210	83.04	76.02	77.05	70.03
240	85.09	81.30	80.50	79.09

Fig. I: FTIR spectra of Physical mixture of drugs and polymer

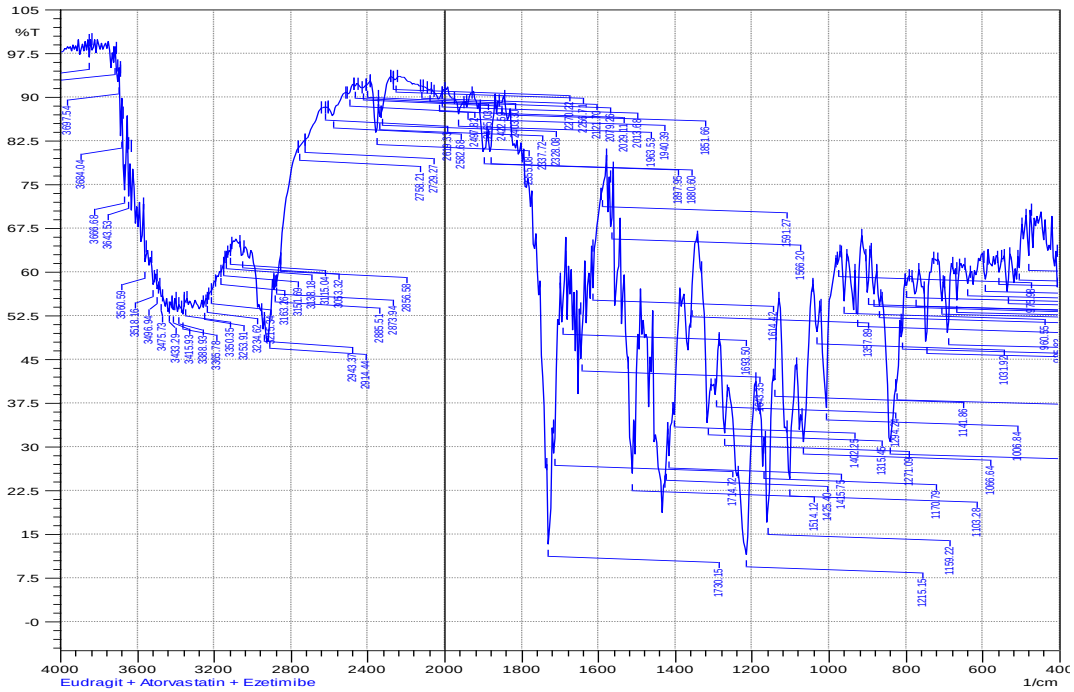


Fig. II: Scanning Electron Micrograph of Atorvastatin calcium and Ezetimibe loaded nanoparticles (F3)

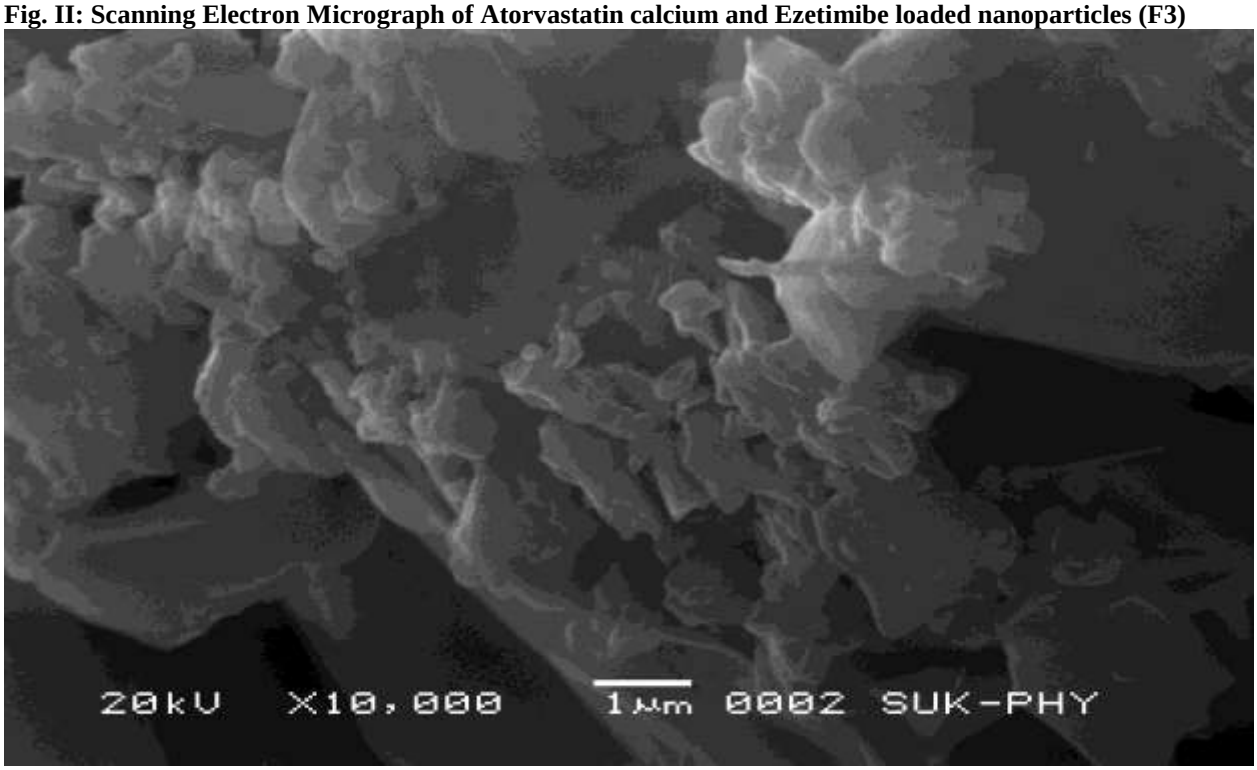


Fig. III: Zetapotential of optimized formulation (F3)

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 15.3	Peak 1:	15.3	100.0	7.06
Zeta Deviation (mV): 7.06	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.810	Peak 3:	0.00	0.0	0.00

Result quality : Good

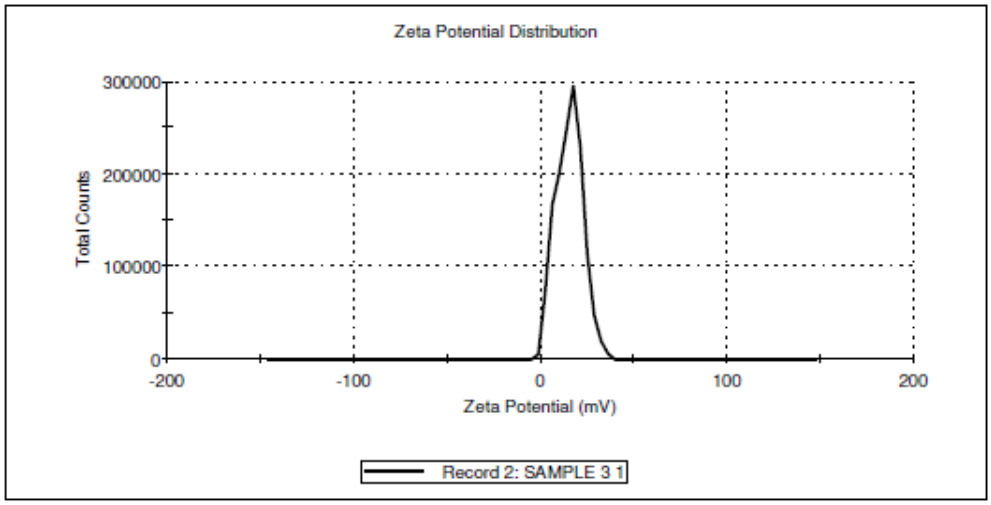
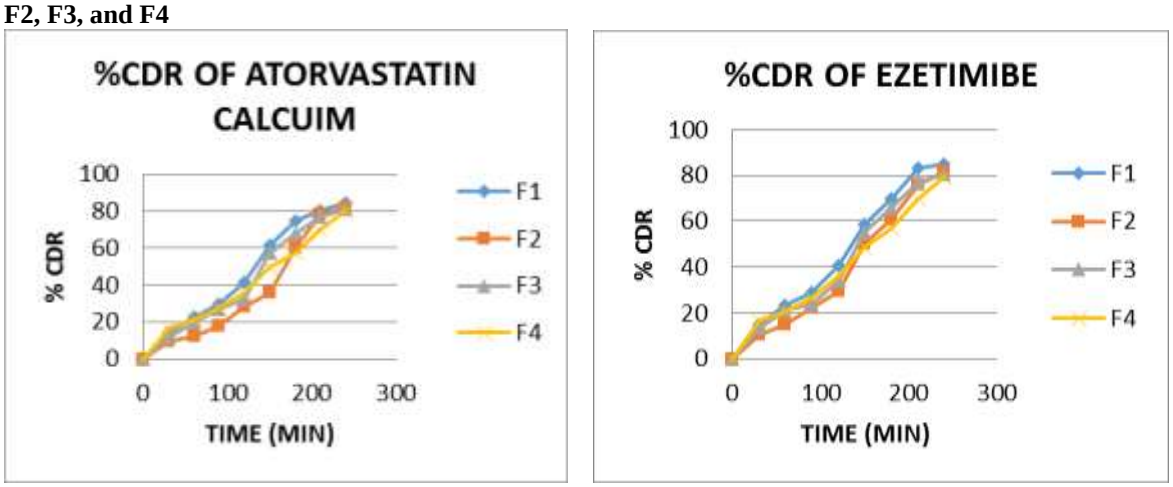


Fig. IV: Comparitive *in vitro* drug release profile of Atorvastatin calcuim and Ezetimibe nanoparticles F1, F2, F3, and F4



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