Formulation, *In Vitro* Evaluation and *In Vivo* Evaluation of Atorvastatin Pulsatile Drug Delivery System

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ABSTRACT*:*

The goal of this study is to develop a pulsatile drug delivery system depending on the core tablet coated with the inner swelling layer and outer rupturable layer. The main core has Atorvastatin calcium with excipients like microcrystalline cellulose and spray-dried lactose. The core tablet is then coated with cross-carmellose sodium as the inner swellable layer followed by a coating of ethyl cellulose as a rupturable layer. Evaluation of prepared tablets was done by %water uptake studies and % cumulative drug release. A lyophilic particulate material, magnesium stearate was included in the formulation which reduces the mechanical strength as well as lag time and the lag time of dosage form can also retarded with an increasing quantity of swelling polymer. The uptake of water by the tablet is decreased by increasing the polymer coating.

KEYWORDS: Atorvastatin calcium, Ethylcellulose, Croscarmellose sodium, Lag time.

Introduction:

For most of the dosage forms, a Chrono therapeutic drug delivery system is very beneficial as these dosage forms release the drug after a certain lag time [1, 2]. These types of delivery systems can be used to treat sequential-behaving diseases like asthma, arthritis, CVS disorders, diabetic mellitus, and hypercholesterolemia. In these cases, the delivery system should be designed to adaptable to the circadian rhythms of diseases. [3, 4]. Most of the pulsatile drug delivery systems are based on a core drug coated with an erodible layer which increases the lag time of the drug. Coating of the core drug also includes a swellable layer before the erodible layer is made, this will increase the efficacy of the drug release pattern. [7-12]

Design of an optimal dosage form for a drug rest on factors corresponding to, the bioavailability of the drug, degree and mechanism of metabolism, and site of absorption. [3, 4] Most often available and conventional dosage forms are in oral form because it offers immediate release of the drug after ingestion another common approach apart from conventional dosage forms another dosage form viz, extended or sustained release dosage forms are available, which will improve the general patient compliance and acceptability. [13, 14]

The main aim of this study is to develop and evaluate the pulsatile drug delivery system of Atorvastatin calcium, which has a core of API coated with swellable and rupturable layers, which will delay the lag time, thereby achieving Chrono modulated form of the drug. Polymers used in rupturable layers play an important role in achieving control of lag time.

Materials and Methodology:

Materials:

Atorvastatin calcium was obtained as a gift sample from Sri Krishna Pharmaceuticals, Hyderabad (TS). Microcrystalline cellulose and HPMC K 100M were obtained from the drug store of SVCP, Tirupati (AP), sodium starch glycolate and spray-dried lactose purchased from Madhav Labs, Hyderabad (TS).

Methodology:

Preparation of core tablets:

Core tablets were prepared to contain 15% by weight Atorvastatin calcium, 50% by weight spray dried lactose, 34% by weight microcrystalline cellulose, and 1% by weight magnesium stearate. All the ingredients were blended and prepared by direct compression. An 8mm punching die set was used to obtain 150mg of tablet weight with 80N of crushing strength. The friability of prepared tablets was measured using Roche Friabilator and was found to be less than 0.05%. [8-10]

Preparation of coating dispersion:

Swelling layer (Inner coating):

The swelling layer was prepared with Croscarmellose sodium (Ac-Di-Sol) using PVP as a binder Croscarmellose sodium and PVP were taken in a ratio of 6:1, respectively. PVP was dissolved in ethanol (96% v/v) and stirred until a clear solution appears. Croscarmellose sodium was dispersed into the above-prepared solution and agitated till a homogenous solution appears. The Pan coating method is used for the coating of core tablets until the required levels of swelling polymer are coated onto the tablet core.[9]

Rupturable layer (Outer coating):

The outer rupturable layer was prepared by dissolving ethyl cellulose in 96%v/v of ethanol and stirring until a clear solution appears. Using dibutyl maleate as a plasticizer (5%w/w depends on the amount of the polymer used for coating). The plasticizer and the prepared ethanolic ethyl cellulose were agitated together for 30 mins before coating.[9]

Coating of the tablets:[11]

For the swelling layer (inner coating) pan coating apparatus was used, and tablets were weighed in the middle of the coating process to verify the levels of polymer coated. The coating was continued until the required amount of swelling polymer coated the tablets. Coated tablets were left aside for curing at 38-40°C overnight. After curing the tablets were again coated till the target weight was achieved. Then they were kept for rest and cured in an oven at 40°C. Final enteric coating (rupturable layer) was completed and the final tablets were left for fluidization for 10 minutes and cured in an oven at 40°C.

Table 1. Processing Parameters

S.No.	Parameter	Value
1	Bed temperature	35-40°C
2	Suspension spray (gm/min)	5-7
3	Spray time (min)	45-50
4	Spray nozzle (mm)	1
5	Spray pressure (kg/cm^2)	4
6	Pan speed	20
7	Drying in equipment	10

Table 2. Working Formula

S.No.	Polymer	F1	F2	F3	F4	F5	F6
1	Croscarmellose sodium	8%	-	-	10%	-	-
2	Sodium starch glycolate	-	8%	-	-	10%	-
3	HPMC	-	-	8%	-	-	10%
4	Ethylcellulose	4%	4%	4%	4%	4%	4%

Table 3. Quantity of Polymers

S.No.	Ingredients	Quantity (mg)
1	Atorvastatin calcium	20
2	Spray dried lactose	80
3	Micro crystalline cellulose	48
4	Magnesium stearate	2
5	Average wt. of tablet	150

Evaluating parameters:

Lag time:

The process for determining the lag time was the same as for the dissolution test, except the lag time was recorded at the time point when the outer coat of the pulsatile tablet was ruptured and removed.[19]

Weight variation:

The average weight of all core tablet formulations and press-coated tablets was found to be within the British Pharmacopoeia, 2016 specified limit.[15]

Friability Test:

The friability of all the tablets analysed was assessed using a Roche friabilator.[16]

The hardness of the Tablet:

The hardness of a tablet indicates its resistance to capping, abrasion, or breakage under storage, temperature, and handling circumstances before use. hard of a tablet is the level of power necessary to break it using a given instrument. The hardness test was used to assess the Hardness of ten tablets (at random). Hardness is measured in kg/cm^2.[17]

Drug content:

Ten tablets were powdered and weighed equivalent to 20 mg of Atorvastatin calcium. The weighed amount was transferred to a 100 ml volumetric flask with having few ml of methanol and shaken well till a clear solution appeared, then make up the remaining volume with methanol. Pipette out 1ml from the above solution to a 10ml volumetric flask and make up the volume with methanol. From this solution pipette out 1.0 ml, 2.0ml, 3.0ml, 4.0 ml, and 5.0 ml into 10 ml volumetric flasks separately and makeup with methanol. Absorbance was measured at 245 nm using methanol as a blank. [10]

In-Vitro dissolution studies:

Dissolution studies were carried out using a USP type I dissolution apparatus under prescribed conditions (Temperature 37±0.5°C, 100 RPM). HCl with pH 1.2 was used as an artificial gastric fluid and pH 6.8 phosphate buffer was used as the intestinal fluid.[9]

Water uptake studies:

The coated tablets were weighed accurately and immersed in an artificial colonic medium of pH 5.4 in a USP type I apparatus with 100 rpm. All parameters are maintained like in the in-vitro dissolution study. Tablets were taken out from the medium at predetermined time intervals, and the tablets were then washed with distilled water to remove buffer solution from the tablets and blotted with lint-free tissue paper. The weight of the tablets was noted down before and after drying.

Percentage water uptake is calculated using the following formula:

$$\% \text{ Water uptake} = \frac{W(t) - W(d)}{W(d)}$$

Here W (t) referred to the weight of the tablets removed at time t.

W (d) referred to the weight of the tablets after drying at time t [10, 11]

In vitro Buoyancy Study:

Using the paddle method, the buoyancy ability of microsphere ads was determined (USP apparatus II). 50 microspheres were spun at 50 rpm for 12 h in a dissolving vessel containing 500 ml of simulated stomach fluid (pH 1.2). The microspheres' ability to float was evaluated visually.[18]

In vivo study:

The study contained 12 male New Zealand white rabbits weighing 2-3 kg and aged 10-14 weeks. They were fed a regular meal and given water, and they were kept in a 12- 12 h light-dark cycle. The protocol for the study was approved by the institutional animal ethical committee (CPCSEA/SVCP/IAEC/I-016/2019-20 dt 29.05.2020). The animals fasted for 24 hours before the investigation. First, the dorsal surface of the ear pinna was depilated by animal hair. The experimental animals were divided into three groups of four each. The dose was calculated from the formula and samples were administered orally to groups 1,2, and 3 respectively.

$$\text{Rabbit dose} = \frac{\text{total dose}(20 \text{ mg in humans}) * 0.07 \text{ (for 1.5 kg rabbit)}}{1.5}$$

At various times, 1 mL of blood was taken into heparinized Pipetted tubes. Blood samples were separated by centrifugation at 1500 rpm and kept at 4 °C. The plasma drug concentration at 240 nm was calculated using a validated HPLC technique. It was outfitted with an Inspire C (18), (250 4.6 mm, 5 m), pH 4.8 PBS, and acetonitrile (42:58 v/v) as mobile phase, a flow rate of 1 mL/min, and a retention period of 2.1 minutes. The pharmacokinetic parameters were defined and statistically compared using analysis of variance (ANOVA) and the T-test test for column comparison. P 0.05 was regarded as statistically significant. The mean and standard error mean are used to express the results.[20]

Results and Discussion:

In vitro Buoyancy Study:

In less than a minute, all of the formulations began to float on the medium and continued to float all through the dissolution trials.

Compatibility studies:

FT-IR studies were carried out to investigate the compatibility between API and other excipients used in the formulation. FT-IR studies proved that the drug was compatible with used excipients as wave numbers are matched with the drug excipient mixture.[10]

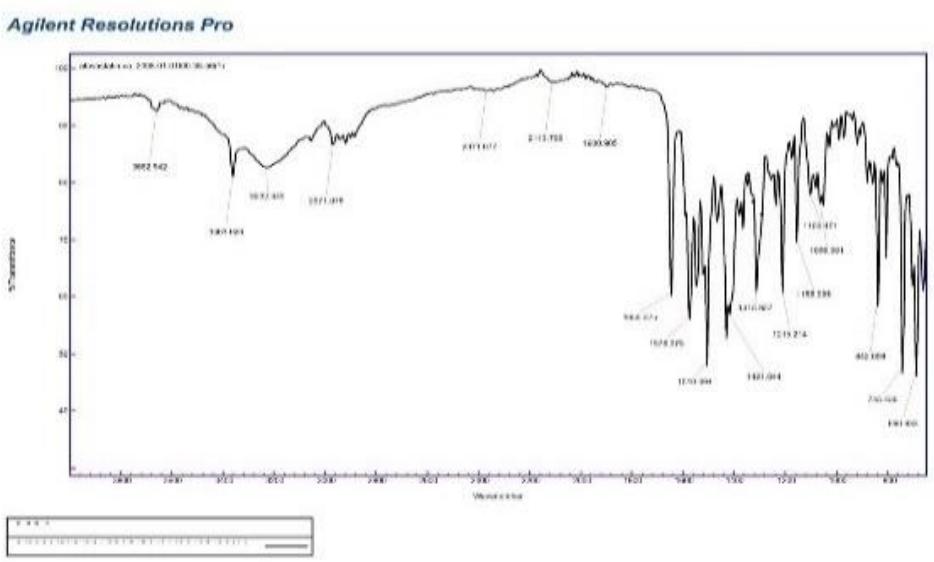


Figure 1. Atorvastatin calcium Sodium

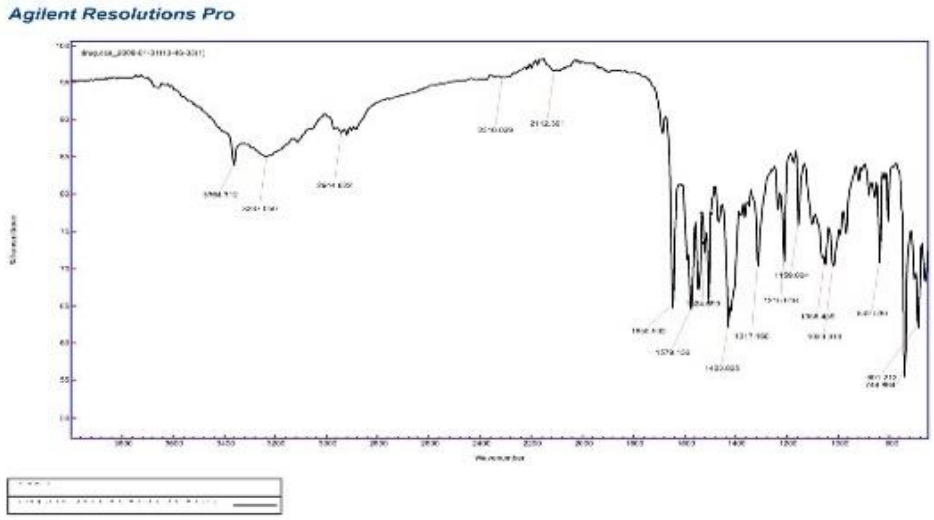


Figure 2. Atorvastatin calcium+Croscarmellose sodium

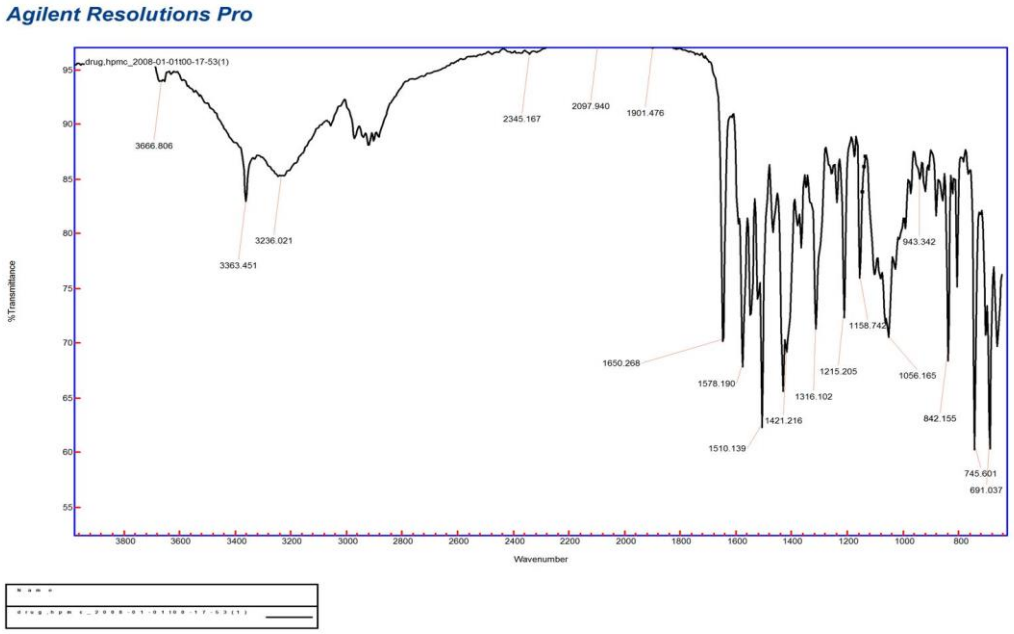


Figure 3. Atorvastatin calcium + HPMC

Precompression parameters:

Results of pre-compression studies showed that the angle of repose of blended powder of the formulation showed as29.50 indicating a good flow property and Carr's index is 18.75, indicating as compressibility is possible. [10,11]

Table 4: Pre-compression parameters of blended powder

Parameters	Readings
Bulk density	0.65± 0.02
Tapped density	0.80±0.25
Carr's Index	18.75±1
Hausner ratio	1.3±0.46
Angle of repose	29.50±1.62

± standard deviation (n=3)

Post-compression evaluations:

Tablets from all six formulations were subjected to thickness, hardness, uniformity of weight, friability, and drug content uniformity. The thickness of the tablets is almost the same. The percentage friability of the tablets was found to be below 1. Weight variation test and other parameters were within the prescribed limits as per official requirements and were found to be 97.1±1 to 98±1 mg of l Atorvastatin calcium as per label.[10]

Table 5: Thickness of coated tablets

Formulation	F1	F2	F3	F4	F5	F6
Thickness	7.4±0.3	7.4±0.3	7.5±0.3	7.36±0.3	7.2±0.3	7.4±0.3

Table 6: Post-compression parameters of F1-F6

Parameters	Core tablet	F1	F2	F3	F4	F5	F6
Weight variation	148.4±0.5	152.8±0.5	153.9±0.5	152.4±0.5	154.5±0.5	152.1±0.5	152.2±0.5
Friability	0.86%	0.68%	0.79%	0.796%	0.81%	0.64%	0.78%
Hardness (kg/cm^2)	4.4	5.3	5.1	4.6	4.4	5.2	5.3
Content uniformity	97.4±1	97.6±1	97.9±1	97.6±1	98.4±1	97.4±1	96.6±1
Disintegration time(sec)	10	669	552	497	529	532	469

± standard deviation (n=3)

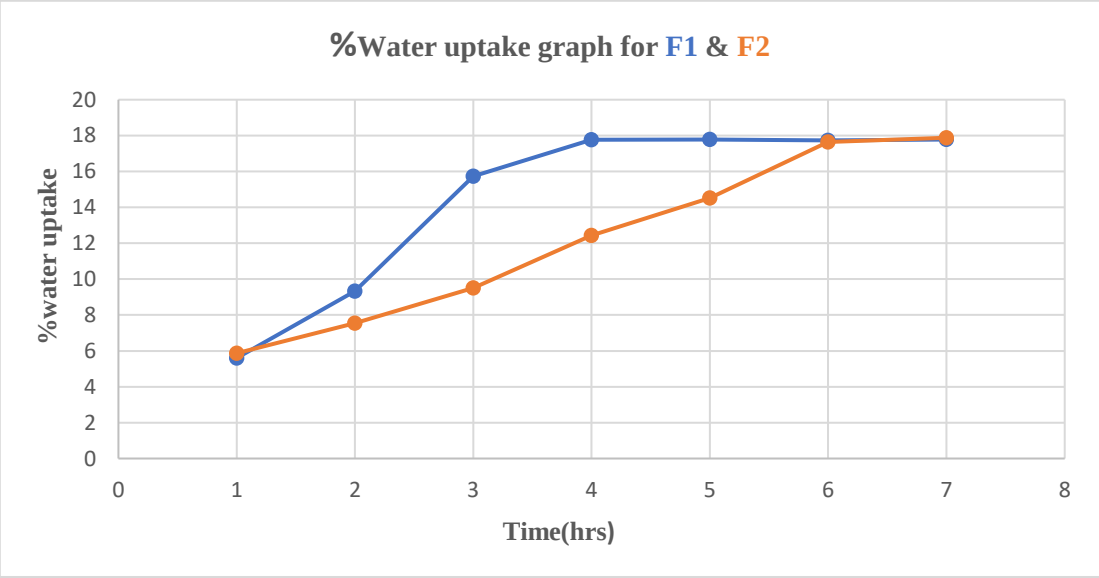
Water uptake studies:

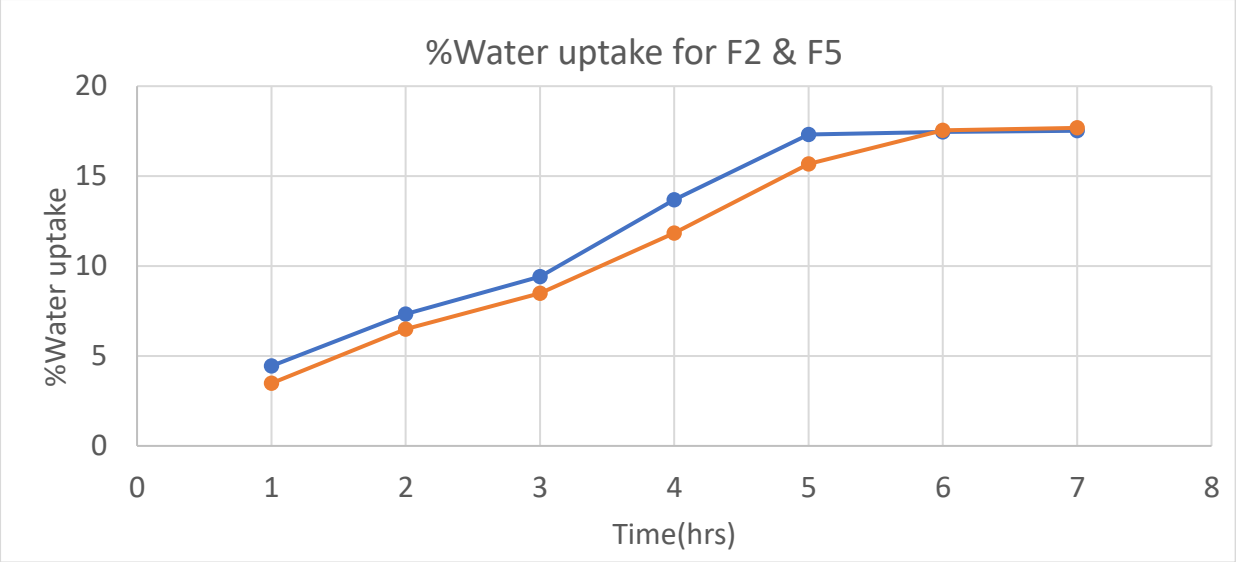
Atorvastatin calcium core tablets coated with both the inner swelling layer and the outer rupturable layer. Water uptake studies were determined in a comparative method generally in pairs i.e., F1 & F3, F2 & F5 and F3 & F6**.** The results were depicted clearly in the following table7.

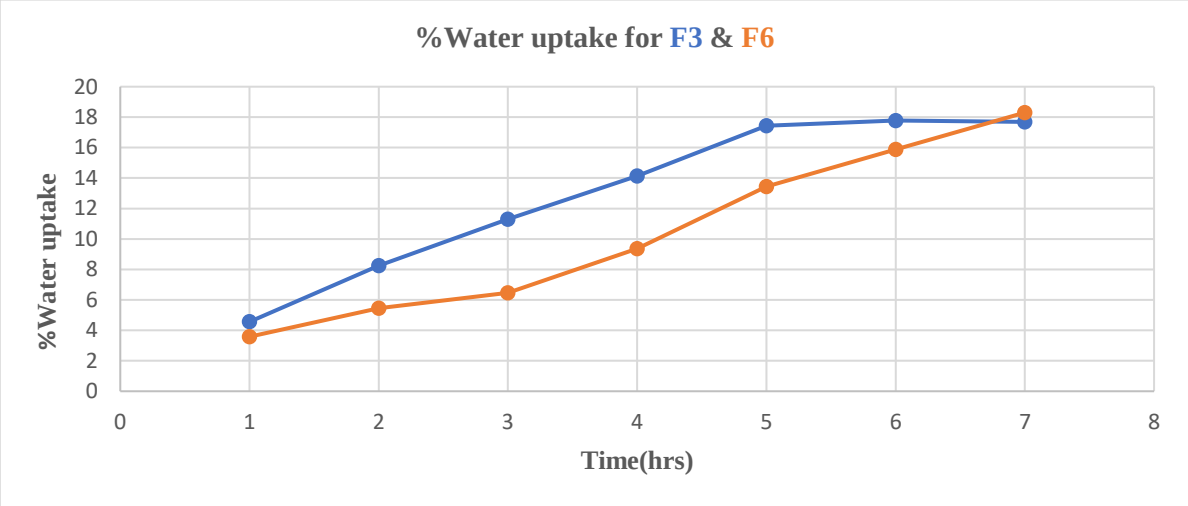
Table 7: Impact of outer coat on %water uptake

Time (hrs.)	F1	F2	F3	F4	F5	F6
1	5.6 ±0.5	4.44±0.4	4.56±0.45	5.87±0.5	3.48±0.25	3.58±0.3
2	9.32 ±0.8	7.33±0.7	8.24±0.8	7.54±0.75	6.50±0.5	5.45±0.0.45
3	15.74 ±1	9.42±1	11.29±1	9.50±1	8.48±0.85	6.47±0.5
4	17.76 ±1	13.69±1	14.14±1	12.44±1	11.83±1	9.36±0.85
5	17.78 ±1	17.32±1	17.44±1	14.52±1	15.68±1	13.44±1
6	17.74 ±1	17.46±1	17.78±1	17.64±1	17.54±1	15.88±1
7	17.78 ±1	17.52±1	17.68±1	17.88±1	17.68±1	18.30±1

± standard deviation (n=3)







Lag time:

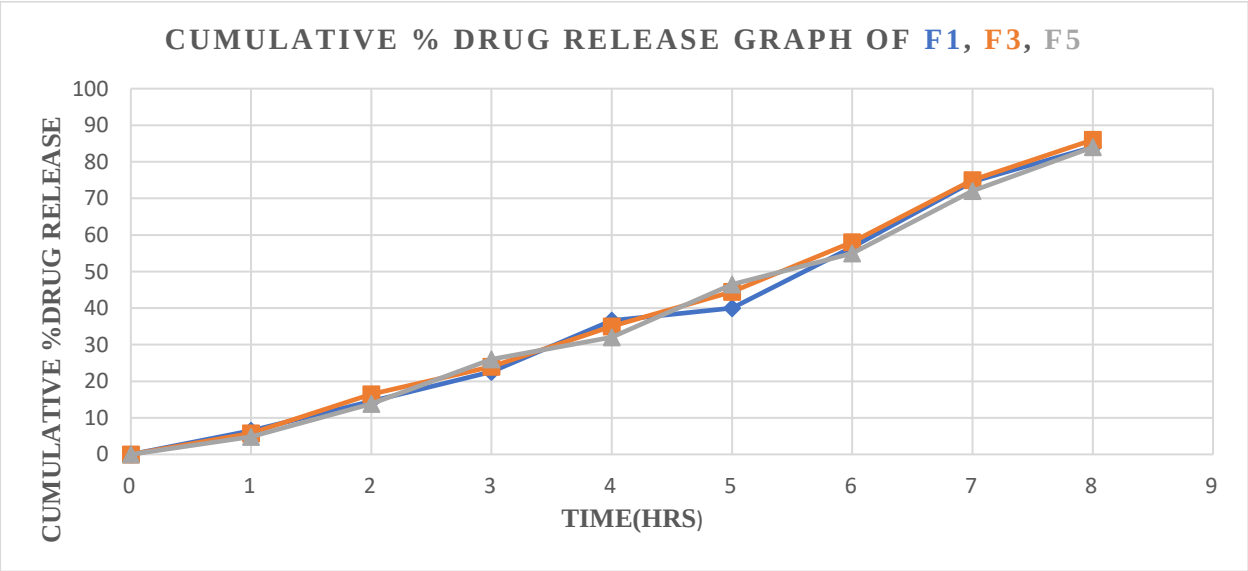
The time at which the burst release of the drug happened for coated tablets was studied, and the results were presented in (Table). Since this actual fixed lag time was 7 hours, the tablet must be ruptured at or after the 7th hour and the medicine must be released completely within 8 hours. The formulation F5 met the requirement. The remaining formulations failed to show the required lag time, which was either less than the lag time (F1, F2, F3 and F4) or more than the lag time, which was >8h (F6). As a result, these formulations were not chosen for further research.

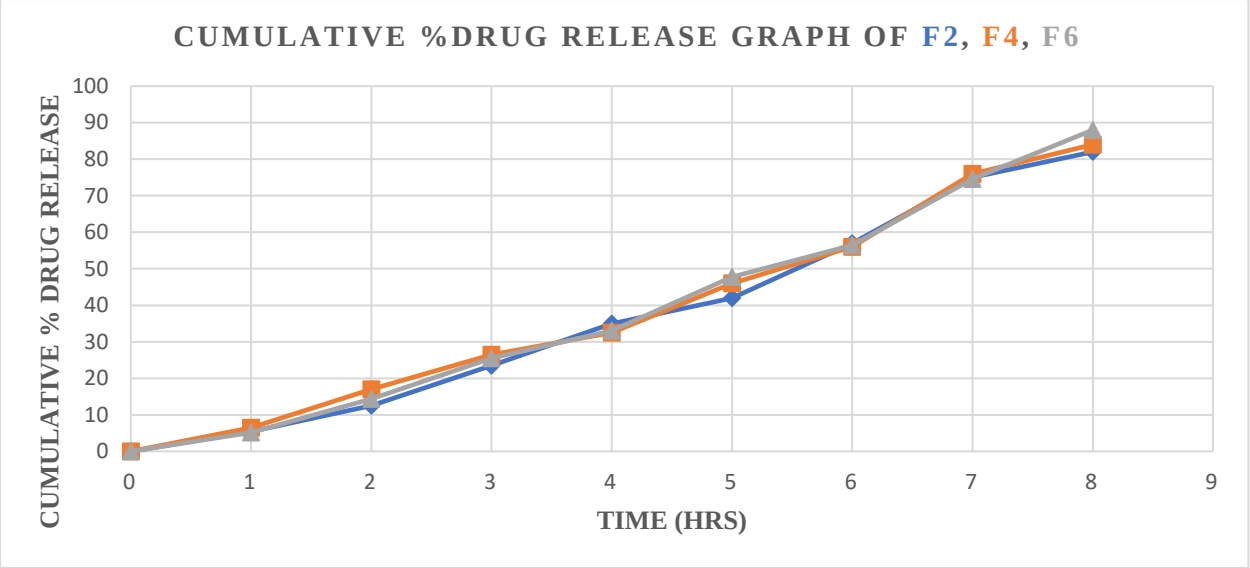
Table 7: Lag time

Formulation	Lag time (h)
F1	3.22
F2	3.28
F3	4.28
F4	6.52
F5	7.00
F6	8.95

In-Vitro release studies:

The percentage cumulative drug release of all six formulations was determined by invitro dissolution studies using USP type II dissolution parameters. The conditions used are similar to those mentioned above. *In-Vitro* dissolution profiles for 8 hours study showed that formulations have good sustaining efficiency. During the process, it was evident that the enteric coat i.e., the outer coat was intact in the 1.2 pH but dissolved in intestinal pH. As a result, pores are formed on the membrane and water is allowed into the tablet leading to swelling of the inner polymer layer which helps in building pressure inside and breaking the outer polymer layer at pH 6.8. In all formulations, the release of the drug from the dosage form in pH 1.2 was negligible and proved the efficiency of the dosage form. Comparative %cumulative drug release was depicted in the graphs below.





In- vivo evaluation:

Table 8 shows the pharmacokinetic data collected from a validated HPLC method used to determine drug concentration in plasma samples. The pharmacokinetic parameters t12, Ke, and Vd for F-2 pellets were enhanced by 1.54, 6.19, and 2.26 times, respectively, according to the results in the table. When contrasted to F1, the bioavailability parameters Cmax, Tmax, AUC, and Ka of F-2 were raised by 3.21, 1.28, 2.45, and 1.25 times, respectively. In the case of F-5 pellets, pharmacokinetic parameters increased 7.66, 41.46, and 2.51 times with higher bioavailability. The administration of dosage in different ways and at different to rabbits resulted in increased oral absorption of nifedipine. The catered formulation was used for micron-sized quick-release pellets with traditional drug release.

Table 8: Pharmacokinetic data

Pharmacokinetic Parameters	F1	F2	F5	Test of significance
C_{max} (ng/mL)	245 ± 96	819 ± 133	359.8 ± 106	S
T_{max} (h)	3.8 ± 9.2	4.9 ± 2.9	16.0 ± 2.9	S
$t_{½}$ (h)	2.2 ± 1.9	3.075± 1.5	14.39 ± 2.8	S
K_{el} (h^{-1})	0.187 ± 0.015	1.222 ± 0.078	8.220 ± 1.48	S
V_d (l/h)	13.1 ± 1.1	28.9 ± 6.9	35.0 ± 6.2	S

Pharmacokinetic Parameters	F1	F2	F5	Test of significance
$AUC_{0–8}$ (ng h mL^{-1})	1839 ± 122.9	4490 ± 168.8	–	S
$AUC_{0–18}$ (ng h mL^{-1})	2549 ± 149.2	–	3519 ± 112.2	S
Ka (h^{-1})	0.72 ± 0.15	0.87 ± 0.29	–	S

± standard deviation (n=3), S: Significant

Rupture test:

Lag time was determined by using a rupture test and was tested by USP type II apparatus and all the parameters were the same mentioned in dissolution studies. The time at which the outer polymer layer started to erode is called lag time.

Formulation no.	F1	F2	F3	F4	F5	F6
Rupture time (hrs)	4	5.6	4.4	4.1	5.2	4.5

Table 9: Rupture test of all formulations

Conclusion:

Atorvastatin calcium tablets of pulsatile delivery type were developed and evaluated successfully. The delivery system released the drug immediately after the lag time. The lag time of the dosage form can be altered by varying quantities of the polymers used in the coating.

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