P.V.S.R.Chandra Sekhar /J. Pharm. Sci. & Res. Vol. 11(8), 2019, 2820-2826

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Formulation and Evaluation of Atorvastatin as Pulsatile Drug Delivery System by Using Pulsincap Technique

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Abstract

The objective of the present study was to design and evaluate modified pulsincap of Atorvastatin according to circadian rhythm using formaldehyde vapours for cross-linking to make capsule body water insoluble and hydrogel plug to achieve a predetermined lag time of ***5hrs*** for chronotherapy of hypercholesterolemia. The capsule body was made water insoluble by exposing the body to formaldehyde vapours. It was sealed with unhardened cap of the capsule. The solid dispersion was prepared using the inclusion complexation with β-cyclodextrin by co-evaporation method sealed within the capsule body by means of a hydrogel plug. Developed formulation was evaluated for *in-vitro* drug release in pH 1.2 (2 hrs), phosphate buffer pH 7.4 (3 hrs) and phosphate buffer pH 6.8 respectively. Sodium alginate 40mg (F3) showed predetermined lag time of 5 hrs so it was selected as optimized formulation and it has shown the immediate release of the drug after the lag time of about 5 hrs.

Key Words: Circardian ryhtm, pulse in cap, Hydrogel plug.

INTRODUCTION:

Pulsatile drug delivery systems (PDDS) have attracted attraction because of their multiple benefits over conventional dosage forms. They deliver the drug at the right time, at the right site of action and in the right amount, which provides more benefit than conventional dosages and increased patient compliance.

Pulsatile drug delivery [1-4] is defined as the rapid and transient release of certain amount of molecules within a short time period immediately after a predetermined off-released period, i.e., lag time. These systems are designed according to the circadian rhythm of the body, and the drug is released rapidly and completely as a pulse after a lag time. These products follow the sigmoid release profile characterized by a time period. These systems are beneficial for drugs with chronopharmacological behavior, where nocturnal dosing is required, and for drugs that show the first-pass effect. Pulsatile drug delivery is time and site-specific drug delivery, thus providing spatial and temporal delivery and increasing patient compliance. (Fig 1)

Control release systems for 12-24hrs drug release are not suitable for diseases which follow circadian variation & in such conditions there is a requirement for pulsatile release, because due to 1st pass effect there will be reduction in bio availability of the drug.Drugs with short $t_{1/2}$ need to be administered repeatedly which results in patient non-compliance.In case of chronic treatment, when the drug is given in SR dosage form, continuous exposure of drug to body may lead to adverse effects.

Purpose to formulate Atorvastatin as Pulsincap

- Circadian rhythm occurs during hepatic cholesterol synthesis which is higher during the night than daylight. However the maximal production occurs early in the morning.
- Evening Atorvastatin dose administration is known to reduce the Cmax (rate of absorption) and AUC (extent of absorption) by 30% each.

This undergoes high intestinal clearance and first-pass metabolism, which is the main cause for the low systemic availability

Pulsincap is one of the approaches for pulsatile drug delivery. The Pulsincap was similar in appearance to a hard gelatin capsules, but the main body was water insoluble. It comprises of a water-insoluble capsule body, soluble cap and hydro gel plug (Fig:2)

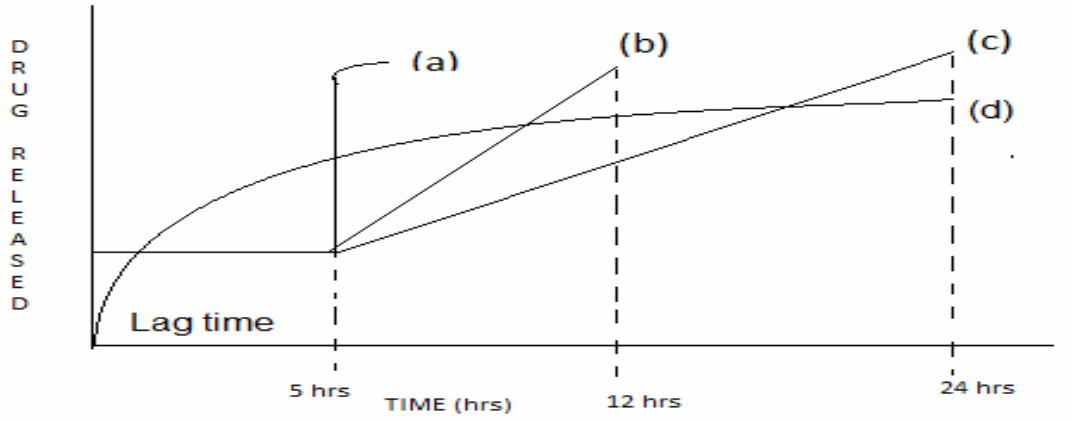


Fig1: Graphical representation of release profile of pulsatile drug delivery system

(a)= sigmoidal release after lag time, (b) = delayed release after lag time, (c) = sustained release after lag time, (d) = extended release without lag time.

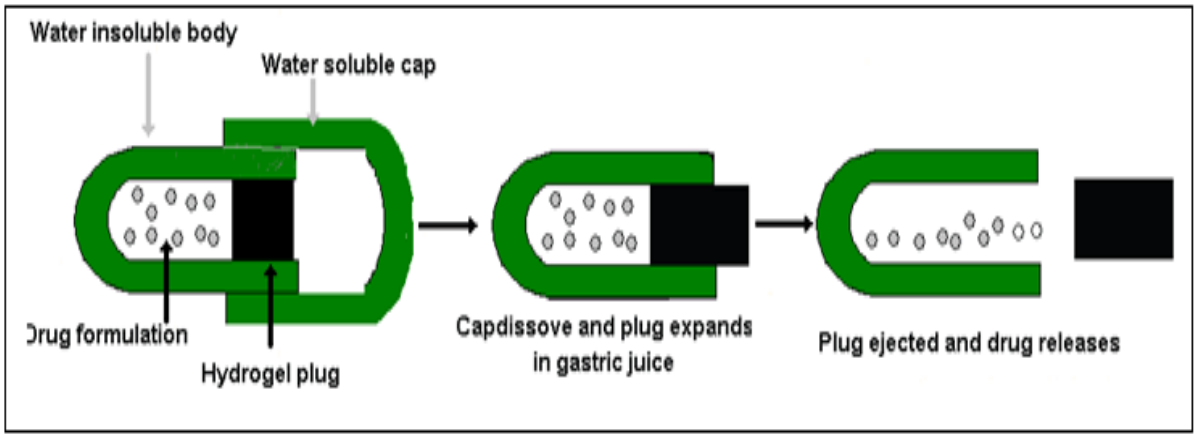


Figure:2

MATERIALS AND METHODS

Materials

Atorvastatin as drug , Beta cyclodextrin as solubility enhancer and the polymers HPMC, Methyl cellulose, Sodium Alginate,Ethyl cellulose Xanthan gum as plug formers

Methodology [5-12]

1. Calibration Curve for Atorvastatin:

Stock solution of 1000µg/ml solution of Atorvastatin was prepared with 0.1N HCl/pH 7.4 phosphate buffer/pH 6.8 phosphate buffer. Different aliquots were withdrawn from the standard stock solution and diluted with appropriate quantity of buffers to get a series of concentrations ranging from 4 to 24µg/ml. And the absorbances of the solutions were measured by using UV-visible spectrophotometer and the calibration curve was prepared by plotting absorbance versus concentration.

2. Preparation of Buffers:

2.1. Preparation of 0.1 N Hydrochloric acid

To 8.5 ml of concentrated Hydrochloric acid, sufficient amount of distilled water was added to adjust the volume 1000ml

2.2. Preparation of pH 7.4 phosphate buffer:

6.805g of Monobasic potassium phosphate and 1.564g of NaOH were accurately weighed and taken in to a 1000ml volumetric flask and the volume was made up to the mark with distilled water.

2.3. Preparation of pH 6.8 phosphate buffer:

6.805g of Monobasic potassium phosphate and 0.896g of NaOH were accurately weighed and taken in to a 1000ml volumetric flask and the volume was made up to the mark with distilled water.

3. Preparation of Cross-Linked Gelatin Capsules:

The '2' sized hard gelatin capsules; about 50 in number were taken. The bodies of the capsules were then placed on a wire mesh. 25ml of 15% v/v formaldehyde was taken into a desiccators and potassium permanganate was added to it to generate formalin vapours. The reaction was carried out for 12 hours. After which the bodies were removed and dried at 50^0C for 30 minutes to ensure completion of reaction between gelatin and formaldehyde vapour. The bodies were dried at room temperature to facilitate removal of residual formaldehyde.

4. Tests for Formaldehyde Treated Empty Capsules:

4.1 Physical tests:

Identification attributes: The size '2' capsule were one with a blue cap and white colored body. They were lockable type, odorless, softy and sticky when treated with wet fingers. After formaldehyde treatment, there were no significant changes in the capsules. They were non-tacky when touched with wet fingers.

Visual defect: In about 50 capsule bodies treated with formaldehyde, about five were found to be shrunk or distorted.

Dimensions: Variations in dimensions between formaldehyde, treated and untreated capsules were studied. The length and diameter of the capsules were measured before and after formaldehyde treatment, using vernier caliper.

4.2 Chemical test:

Qualitative test for free formaldehyde:

Standard formaldehyde solution used is formaldehyde solution (0.002 w/v) and sample solution is formaldehyde treated bodies (about 25 in number) were cut into small pieces and taken into a beaker containing distilled water. This was stirred for 1 hrs with a magnetic stirrer, to solubilise the free formaldehyde. The solution was then filtered into a 50 ml volumetric flask, washed with distilled water and volume was made up to 50 ml with the washings. 1ml of sample solution, 9 ml of water was added. One ml of resulting solution was taken into a test tube and mixed with 4 ml of water and 5 ml of acetone reagent. The test tube was warmed in a water bath at 40ºC and allowed to stand for 40 min. The solution was not more intensely colored than a reference solution prepared at the same time and in the same manner using 1ml of standard solution in place of the sample solution. The comparison should be made by examining tubes down their vertical axis.

5. Solubility Enhancement of Atorvastatin:

*5.1. Preparation of Solid dispersion by using solvent evaporation***:**

Drug and polymer **PEG4000** in the ratio of **1:5** were taken into a china dish and mixed thoroughly. Then methanol as solvent was added. Continuous stirring was ensured for proper mixing of drug and polymer and solvent was allowed to evaporate. Then the solidified mass was crushed and sieved through 44 no. sieve and stored in a desiccator.

5.2. Preparation of inclusion complex by co-evaporation method:

Atorvastatin and **β-cyclodextrin** in the ratio of **1:1** were accurately weighed and dissolved in methanol and water respectively. Then the solutions were mixed thoroughly and solvents were evaporated by controlled heating at 45^0C - 50^0C.Then dried mass were pulverized and sieved through sieve no.44 and stored in a desiccator.

5.3 In vitro release profile of Atorvastatin solid dispersion:

In vitro dissolution studies were performed for selected solid dispersion. The following conditions were maintained for the dissolution process:

Apparatus: Paddle type. ,Temperature: 37±0.5^{0}C ,RPM: 75 ,Dissolution medium: Ph 6.8 Phosphate buffer ,Volume of medium: 900 ml ,Sample volume: 5 ml withdrawn and replaced with 5 ml of distilled water. The withdrawn samples were analyzed at 241nm, by UV-visible spectrophotometer and the cumulative percentage release was calculated over the sampling times.

6. Preparation of Hydrogel Plug:

Plug for sealing the capsule body was prepared by compressing required amount of polymers like sodium alginate KO5/ Xanthan gum /HPMC KO5/methyl cellulose using 6mm punches and dies on rotary tablet press keeping varying thickness and hardness values of tablet plug. This plug was then fitted into the body of cross-linked gelatin capsule containing solid dispersion equivalent to 10 mg of Atorvastatin and then the cap was closed.

7. Designing of pulsincap capsules:

Pulsincap dosage form was a capsule which consists of a water insoluble body and a water soluble cap. The drug formulation (solid dispersion equivalent to 10mg) was sealed within the capsule body by means of a hydrogel plug. Different formulations are prepared and with various polymers as shown in the **Table 3**. When the pulsincap was swallowed, the water soluble cap dissolves in the gastric juice and the exposed hydrogel plug begins to swell. At predetermined time (5hrs) after ingestion, the swollen plug was ejected out and the encapsulated drug formulation was then released into the colon, where it is dissolved and then absorbed into blood stream. In the present study, capsule bodies which were hardened with formaldehyde treatment for 12hrs were used for the preparation of pulsincaps. It was sealed with unhardened cap of the capsule. The solid dispersion was prepared using the inclusion complexation with β-cyclodextrin by co-evaporation method sealed within the capsule body by means of a hydrogel plug.

8. Evaluation:

8.1. Physicochemical Characterization of Hydrogel Plug:

Hydrogel Plugs were studied for hardness, friability, weight variation, lag time and Swelling Index.

8.2. Determination of Swelling Index of Hydrogel Plug:

Hydrogel plugs of 100mg were taken and kept immersed in three different pH conditions. Plugs were taken out carefully at 2, 4, 6 hours and their weights were determined accurately.

$$\% \text{ Swelling} = \frac{\text{wet weight} - \text{dry weight}}{\text{Wet weight}} \times 100$$

8.3. Drug content uniformity:

The solid dispersion of Atorvastatin equivalent to 10mg was dissolved in buffer and estimated spectrophotometrically at 241nm.

8.4 Lag time:

Lag time is the total time period after which the plug is ejected out of the capsule body and the drug releases immediately. Lag time was determined visually using phosphate buffer pH 6.8. For lag time determinations USP II paddle apparatus was used. Capsules were tied with the paddle by cotton thread; temperature was maintained at 37°C at 50 rpm.

8.5. In-vitro release profile of pulsatile capsule:

Dissolution studies were carried out by using USP I dissolution test apparatus (basket method). In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used referred to as sequential pH change method. When performing experiments, the pH 1.2 medium was first used for 2 hrs (since the average gastric emptying time is 2 hrs), then removed and the fresh pH 7.4 phosphate buffer was added. After 3 hrs (average small intestinal transit time is 3 hrs), the medium was removed and colonic fluid pH 6.8 buffer was added for subsequent hours.900ml of the dissolution medium was used at each time. Rotation speed was 100 rpm and temperature was maintained at 37±0.5^{o}C. 5ml of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 241nm, by UV-visible spectrophotometer and the cumulative percentage release was calculated over the sampling times.

In this study, an attempt has been made to design, formulate and evaluate Pulsatile drug delivery formulation of Atorvastatin, based on pulsincap approach for the treatment of hypercholesterolemia.

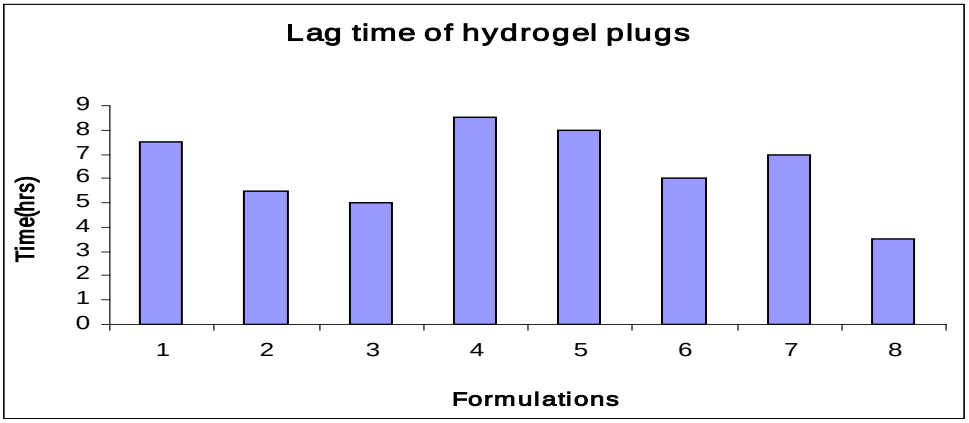
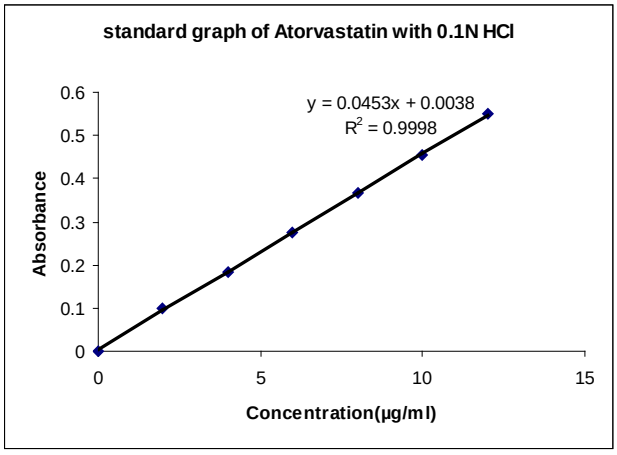


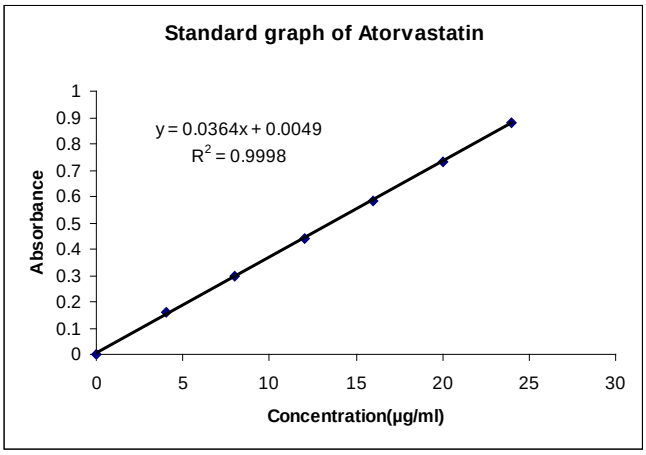
Fig.3: Comparative graph for Lag times of polymer plugs

Table 1: Calibration data of Atorvastatin with 0.1N HCl and Phosphate buffer of pH 6.8 and pH 7.4

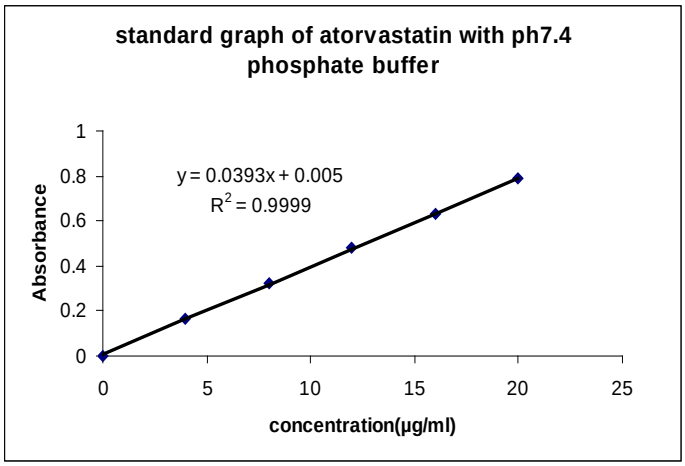
S.No	Conc.(µg/ml)	Absorbance at 245nm		
		0.1N HCl	Phosphate buffer pH6.8	Phosphate buffer of pH7.4
1.	0	0	0	0
2.	2	0.097	0.091	0.086
3.	4	0.186	0.166	0.168
4.	6	0.275	0.242	0.244
5.	8	0.363	0.304	0.323
6.	10	0.454	0.401	0.399
7.	12	0.543	0.453	0.478



Graph : 1 Calibration curve of Atorvastatin with 0.1 N HCl



Graph :2 Calibration curve of Atrovastatin with phosphate buffer of pH 6.8



Graph:3 Calibration curve of Atorvastatin with Phosphate buffer of pH 7.4

RESULTS AND DISCUSSION:

1. Standard graph of Atorvastatin:

The absorbance of a series of solutions having different concentrations of Atorvastatin is given in table 3 – 5 and the **Graph 1-3** shows calibration curves of Atorvastatin which obtained when concentration in µg/ml was plotted

against absorbance. It gave straight line that passes through the origin in pH 1.2, pH 7.4 and pH 6.8 mediums. The correlation coefficient has been determined and found to be 0.9998, 0.9999 and 0.9998 respectively.

2. Solubility Enhancement of Atorvastatin:

Atorvastatin solubility was enhanced by using polymer PEG4000 and β-cyclodextrin. β-cyclodextrin has shown greater solubility when a graph was plotted between cumulative % amount dissolved and time when compared to polymer PEG4000 as shown in the (**Table 2**) & (Graph 4).

3. Preparation and evaluation of Cross-Linked Gelatin Capsules:

Hardening of capsules was done by using formalin vapours and physical and chemical evaluation tests such as identification attributes, visual defects, dimensions and qualitative test for formaldehyde have been conducted as per the procedure and satisfactory results were obtained.

4. Selection of polymer for hydrogel plug:

Water uptake studies were done on different polymers like HPMC KO5, Sodium alginateKO5, Methyl cellulose & Xanthan gum and % swelling was calculated as per the formula and the results were tabulated in (**Table 4**). Among this sodium alginate has shown more swelling index (81.4%).

5. Preparation and evaluation of Hydrogel Plug:

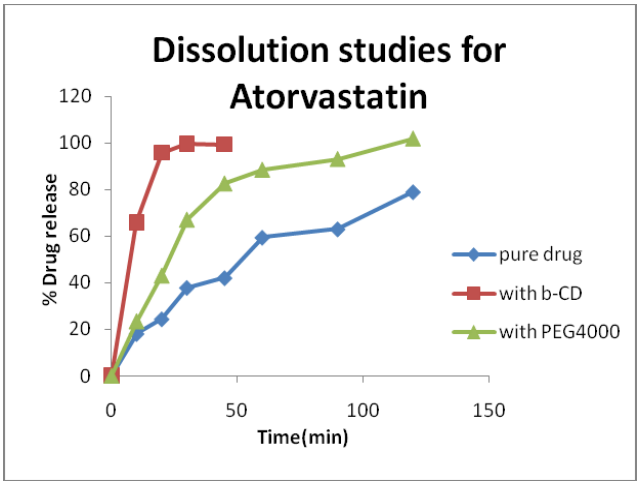
Different Hydrogel plugs (HP1 to HP8) were prepared with varying amounts of polymers and evaluation tests were conducted and the results were tabulated **(Table 5).**

The formulations fitted with the various hydrogel plugs HP1,HP2, HP3, HP4,HP5,HP6 & HP7 shown 5.5%,5.25%,4.5%,6.3%,6.2%,5.91 & 7.75% of drug release respectively at the end of 5th hour (Table).It was observed that weight and hardness of the hydrogel plug was satisfactory to retard the drug release in small intestinal fluid and too ejected out the plug in colonic fluid and releasing the drug into colonic fluid. The prepared hydrogel plugs HP1,HP2, HP3, HP4,HP5,HP6,HP7 & HP8 shown a lag times of 7.5,5.5,5,8.5,8,6,7 & 3.5hrs respectively as shown in the Table 5 & **(Fig. 3)**

Table 2: Dissolution studies for Atorvastatin solubility

S.No.	Time(min)	%Amount dissolved		
		Pure drug	With β-CD	With PEG-4000
1.	10	18	66	23.4
2.	20	24.3	96	43
3.	30	37.8	99.8	67
4.	45	42	99.5	82.5
5.	60	59.4	-	88.4
6.	90	63	-	93
7.	120	79	-	101.8

enhancement mixtures:



Graph:4 Dissolution studies of Atorvastatin Solid Dispersion complexes

Table3: Different formulations of Atorvastatin Pulsatile capsules

Contents	F1	F2	F3	F4	F5	F6	F7	F8
DRUG mixture +Lactose (mg)	20+10	20+10	20+10	20+10	20+10	20+10	20+10	20+10
Sodium alginateKO5 (mg)	75	50	40	-	-	-	50	-
HPMC KO5 (mg)	-	-	-	40	-	-	-	-
XanthangumKO5(mg)	-	-	-	-	40	-	-	-
Methyl cellulose(mg)	-	-	-	-	-	40	-	-
Sodium alginate(low viscous) (mg)	-	-	-	-	-	-	-	70
Ethyl cellulose (mg)	-	-	-	-	-	-	-	30
Lactose (mg)	-	-	-	-	-	-	50	-
Total weight(mg)	105	80	70	70	70	70	130	130

***6. In -Vitro* Drug Release Study:**

During dissolution studies, it was observed that, the cap was dissolved in gastric pH, and then the exposed polymer plug absorbed the surrounding fluid, swelled and released the drug through the swollen matrix. After complete wetting of the plug, it formed a soft mass, which was then easily ejected out of the capsule body; releasing the drug into simulated colonic fluid (pH 6.8 phosphate buffer). From the *In-vitro* drug release studies of device, it was observed that with all formulations except F8 there was absolutely no drug release in simulated gastric fluid(acidic PH 1.2) for 2hrs and in simulated intestinal fluid (PH 7.4 phosphate buffer) for 3hrs. Burst effect was found in colonic medium (PH 6.8 phosphate buffer). *In-vitro release* profiles in colonic medium found to have efficacy for about 4hrs.

Formulations F1, F2&F3 are prepared with sodium alginate K05 in different concentrations (75mg, 50mg & 40mg respectively) depending on water uptake studies (Table3) conducted on different polymers. Among these F3 (i.e. Sodium alginate K05 - 40mg) formulation has shown the required lag time of 5hrs and drug was released up to 4hrs after lag time.

Based on above results, formulations F4, F5&F6 are formulated as same as F3 with remaining polymers such as HPMC KO5 (40mg), XanthangumKO5 (40mg) & Methyl cellulose (40mg) and shown the lag times of 8.5, 8 & 6hrs respectively.

Table 4: Determination of swelling index for different polymers:

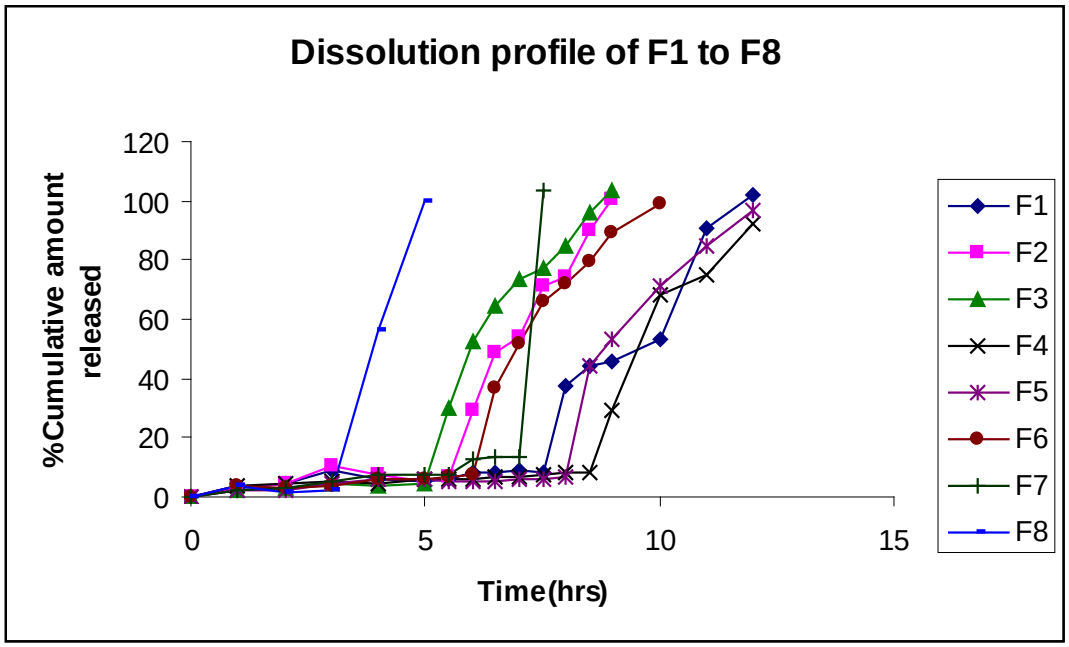
Polymer	***% Swelling***
HPMC KO5	63.6
Sodium alginate KO5	81.4
Methyl cellulose	71
Xanthan gum	64.14

Table 5: Evaluation tests of Hydrogel plugs:

Hydrogel Plug	**Weight(mg)**	**Thickness(mm)**	**Hardness (Kg/Cm2)**	**Lag Time(Hours)**
HP1 (Sodium alginate)	75	2.87	4	7.5
HP2 (Sodium alginate)	50	2.15	2	5.5
HP3 (Sodium alginate)	40	1.78	1.5	5
HP4 (HPMC)	40	1.92	1.5	8.5
HP5 (Xanthan gum)	40	1.74	1.5	8
HP6 (MC)	40	1.66	1.5	6
HP7(sodium alginate+lactose)	100	3.46	3.5	7
HP8 (low viscous SA+EC)	100	3.61	3	3.5

Table 6: Atorvastatin release from pulsatile capsules of formulation F1 to F8:

S.No.	**Time(hrs)**	**%Amount Release**							
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
1.	1	3.25	2.75	2.25	3.5	1.9	3.6	2.5	4
2.	2	4.25	4.5	3	4.2	2.4	2.9	3.25	1.75
3.	3	8.75	10.75	4.25	5.12	4.3	4.1	5.5	2.5
4.	4	5.75	7.5	4	4.5	5.7	5.67	7.25	56.25
5.	5	5.5	5.25	4.5	6.3	6.2	5.91	7.75	99.5
6.	5.5	-	6.5	30	-	-	-	-	-
7.	6	8.25	29	52.75	6.24	5.13	7.81	12.75	-
8.	6.5	-	49	64.75	-	-	37	-	-
9.	7	8.75	54	73.5	6.57	5.89	52	13.75	-
10.	7.5	8.5	71.25	77.25	-	-	66.32	82.75	-
11.	8	37.25	74.25	-	8.25	6.78	72.36	103.75	-
12.	8.5	44.5	-	-	8.13	44	79.80	-	-
13.	9	45.5	100.85	103.75	29.5	53.4	89	-	-
14.	10	53.5	-	-	67.9	71	99.37	-	-
15	11	91	-	-	74.8	84.56	-	-	-
16.	12	102.25	-	-	92	96.5	-	-	-



Graph: 5 Dissolution studies for pulsincap of Atorvastatin (F1-F8)

CONCLUSION:

From the water uptake studies carried out on different polymers, the sodium alginate shows the better swelling nature. From all the performed in vitro drug release studies, formulation F3 i.e. Sodium alginate-40mg shows the required lag time of 5hrs and it was selected as optimized formula. All the evaluation tests such as Determination of Swelling Index of Hydrogel Plug, Drug content uniformity, Lag time, In-vitro release profile of pulsatile capsule, Weight variation, Hardness, were conducted on optimized formulation which has given satisfactory results.

Thus designing of proper pulsatile drug delivery will enhances the patient compliance, optimum drug delivery to the target site and minimizes the undesired effects. It should be pointed that these drug delivery systems are still in the early developmental stage and much research will have to be conducted for such systems become practical clinical alternatives. In the present study, a hard gelatin capsule based pulsatile drug delivery system with rapid drug release after a predetermined lag time (5 hours) was developed. The optimized formulation among all the formulations showed drug release of nearly 90 to 97% within 4 hours after lag time. The lag time was controlled by polymer plug which will be taken at bed time with a programmed start of drug release early in morning hours. The system was produced with approved excipients and standard processes.

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