FORMULATION AND *IN VITRO* EVALUATION OF MODIFIED PULSINCAP OF AMLODIPINE BESYLATE: AN IMPROVED APPROACH FOR THE TREATMENT OF HYPERTENSION

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ABSTRACT

Pulsatile system is gaining a lot of interest as it increases patient compliance by means of providing time and site specific drug delivery system. The aim of the present study was to formulate and evaluate pulsatile drug delivery system of amlodipine besylate based on chronopharmaceutical approach for the treatment of hypertension. The basic design involves the preparation of cross-linked hard gelatin capsules by using formaldehyde. Due to formaldehyde treatment the length, external diameter, thickness, weight of the capsules was increased. Then the drug diluents mixture were prepared and loaded in, which was separated by using hydrogel plugs such as HPMC 50 cP, 100 cP, K100LV, methocel K15, sodium CMC, carbopol 971 and xanthan gum at different amount. Prepared formulations were subjected to *in vitro* drug release studies. From the *in vitro* dissolution studies it was found that by increasing the amount of polymer, release rate was decreased. The release rate was above 90% when we used 50 mg and 75 mg polymer (in each hydrogel plug), but in case of using 100 mg polymer the release rate was 70% to 85% in 10 hr. That means, in 12 hr these formulations can give a satisfactory result which is the most desire in pulsatile drug delivery system. Furthermore, the release data of all formulations were fitted to various mathematical models such as zero order, first order, Korsmeyer Peppas, Higuchi and Hixson-Crowell kinetics. The drug release follows mixed order kinetics and mechanism was found to be non-Fickian diffusion. From the result it was concluded that, all formulations showed compliance with chronotherapeutic objective of hypertension and these modified pulsincap formulations can be a best alternative for high blood pressure patient to avoid multiple dosing. However, further studies can be performed to determine the accurate dosing and better therapeutic effect.

Key words: Pulsatile drug delivery, amlodipine besylate, cross-linked hard gelatin capsule, hydrogel plug.

INTRODUCTION

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in human or animals. Drug delivery systems are engineered technologies for the targeted delivery and/or controlled release of therapeutic agents. For sustained release systems, the oral route of administration has received the most attention with respect to research over various issues. This is because there is more flexibility in dosage regimen for the oral routes than any other routes. The reason that the oral route achieved such popularity is due to its ease of administration and traditional belief that by oral administration the drug is well absorbed.[1] Nowadays, the emphasis of pharmaceutical galenic research is turned towards the development of more efficacious drug delivery systems with already existing molecules rather than going for new drug discovery because of the inherent hurdles posed in drug discovery and development process.[2] But still for many of the drugs, use of such system is not suitable because of a number of reasons. There are many conditions that demand pulsatile release. Sometimes, it is required that the drug should not be released at all during the initial phase of dosage form administration. This condition demands release of drug as a "pulse" after a time lag and such system has to be designed in a way that complete and rapid drug release should follow the lag time. Such systems are known as pulsatile drug delivery systems (PDDS), time-controlled systems, or sigmoidal release systems.[3-6]

Pulsatile drug delivery system delivers drug in rapid and burst manner within a short time period immediately after a programmable lag phase.[7] A pulsatile-release profile is characterized by a time period of no release (lag time) followed by a rapid and complete drug release. PDDS can be classified into site-specific systems in which the drug is released at the desired site within the intestinal tract.[8] That is why pulsatile systems are gaining a lot of interest as they deliver the drug at the right site of action at the right time and in the right amount, thus providing spatial and temporal delivery and increase patient compliance. The release of the

drug as a pulse after a lag time has to be designed in such a way that a rapid and complete drug release follows the lag time. These systems are designed according to the circadian rhythm of the body.

The potential benefits of pulsatile drug delivery have been demonstrated in the management of a number of diseases. PDDS is likely to be successful for diseases such as asthma, myocardial infarction, angina pectoris, peptic ulcer, arthritis, hypertension, hypercholesterolemia as in these diseases particular rhythms in the onset and extent of symptoms are observed.[9]

Cardiovascular diseases such as hypertension and angina, or chest pain, also follow a definite circadian rhythm. Hypertension is increased in the early morning hours. Systolic blood pressure rises approximately 3mm Hg/hr for the first 4-6 hours post-awakening, while the rate of rise of diastolic blood pressure is approximately 2mm Hg/hr. The silent ischemic events showed a circadian pattern with a high density of 34% events occurring between 6 a.m. and noon.[10] The causes for these findings have been suggested to be release of catecholamine, cortisol increase in the platelet aggregation and vascular tone.

Amlodipine besylate is a calcium channel blocking agent with vasodilatory activity. It is mainly used for its antiarrhythmic, antianginal and antihypertensive activity.[11] It is used as the prototype drug and the drug works by slowing down the rate at which calcium moves to heart and walls of blood vessels allowing better blood flow.
It is a dihydropyridine calcium antagonist that inhibits the trans-membrane influx calcium ions into vascular smooth muscle and cardiac muscle.[12] It mainly acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. Also, as a calcium channel blocker amlodipine is expected to inhibit the currents of L-type Cav1.3 channels in the zona glomerulosa of the adrenal cortex, reducing aldosterone production and corroborating to lower blood pressure. Thus the objective of the present study was to prepare a standard pulsatile drug delivery system of amlodipine besylate based on chronopharmaceutical approach for the treatment of hypertension and to evaluate different parameter of the formulations.

MATERIALS & METHODS

Materials:

Amlodipine besylate was a kind gift from ACI Pharmaceuticals Ltd. and the empty capsule shells of size ‘0’ were gifted by Eskayef Bangladesh Limited. HPMC of different grades, sodium CMC, carbopol 971, methocel K15 and xanthan gum were from Loba Chemicals, India.

Formaldehyde treated crossed-linked gelatin capsule:

About 100 hard gelatin capsules of size ‘0’ were taken. Their body was separated from the cap and placed on a wire mesh. Then 25 ml of 37% v/v of formaldehyde solution was taken in a beaker and kept in an empty glass dessicator. 2.5 g of potassium permanganate was added to this. On the top of the beaker a wire mesh was kept and immediately the dessicator was tightly closed and sealed. The body of the capsule was made to react with formaldehyde vapors for 4 hr. Then they were removed and kept on a filter paper and dried for 48 hr in an open atmosphere to facilitate removal of residual formaldehyde. These capsule bodies were rejoined with untreated caps and stored in a polyethene bag for further studies.[13,14]

Formulation of the pulsatile capsules:

Different formulations were prepared using the polymers as hydrogel plugs. The formulations are given in Table 1.

Table 1. Illustration of composition of different formulation

Formulation code	Polymer	Grades	Drug-lactose mixture	Amount of polymer used plug
F-1	HPMC	50cps	150mg	50mg
F-2	HPMC	100 cps	150mg	50mg
F-3	Sodium-CMC	100cps	150mg	50mg
F-4	HPMC	K100LV	150mg	50mg
F-5	Carbopol	971	150mg	50mg
F-6	Methocel	K15	150mg	50mg
F-7	Xanthan gum	Nil	150mg	50mg
F-8	HPMC	50cps	150mg	75mg
F-9	HPMC	100 cps	150mg	75mg
F-10	Sodium-CMC	100cps	150mg	75mg
F-11	HPMC	K100LV	150mg	75mg
F-12	Carbopol	971	150mg	75mg
F-13	Methocel	K15	150mg	75mg
F-14	Xanthan gum	Nil	150mg	75mg
F-15	HPMC	50cps	150mg	100mg
F-16	HPMC	100 cps	150mg	100mg
F-17	Sodium-CMC	100cps	150mg	100mg
F-18	HPMC	K100LV	150mg	100mg
F-19	Carbopol	971	150mg	100mg
F-20	Methocel	K15	150mg	100mg
F-21	Xanthan gum	Nil	150mg	100mg

Filling of capsules

Hard gelatin capsules of size '0' with formalin treated body and untreated cap were taken for filling. Four capsules for each formulation were prepared manually. The cap and body of the known weight of capsule was separated individually by hand. The drug (amlodipine besylate) was filled into the body of capsule; this forms the second dose of the drug. To fill the capsule properly the drug was mixed with an equal amount of lactose first and then 150 mg of the mixture was taken in to the capsule body. The quantity of the polymer as specified in Table 1 for different formulations was weighed and taken above drug-lactose mixture then pressed tightly with a glass plunger; this forms plug 2 of the formulation. Similarly the first dose of drug-lactose mixture was then placed and polymer of plug 1 of same amount was taken in the capsule. Finally the cap was locked and stored in tightly packed container for further studies.[13]

Evaluation of formaldehyde treated capsules

Various physical and chemical tests were carried out simultaneously for both formaldehyde treated and untreated capsules. The identification attributes like color, odor, lock ability, stickiness and shape were checked manually. The size of the capsules, i.e. length, external diameter and thickness was determined by using Vernier calipers.[13]

Qualitative test for free formaldehyde

A suitable volume of formaldehyde solution was diluted with water to give a solution containing 20 µg/ml formaldehyde. After that twenty five formaldehyde treated bodies were cut into small pieces and taken into a beaker containing distilled water (40 ml). This was stirred for 1hr with a magnetic stirrer to solubilize the free formaldehyde. The solution was filtered into a 50 ml volumetric flask, washed with distilled water and volume was made up to 50 ml with washings.

To 1 ml sample solution in a test tube, 4 ml of water and 5 ml of acetyl acetone solution were added; the test tube was placed in a water bath kept at 40^{o}C for 40 min. At the same time reference solution was placed in the same manner using 1ml of standard formaldehyde solution. The sample solution was not more intensely colored than the standard solution inferring that less than 20 µg/ml of free formaldehyde was present in 25 capsules body.[13]

Evaluation of modified pulsincap

In vitro dissolution profile of each formulation was determined by employing USP XXIII apparatus by rotating basket method in colonic fluid pH 6.8 buffer for 10 hr. The dissolution media was maintained at a temperature of 37± 5^{o}C, the speed of rotation of basket maintained were 50 rpm. Pulsing capsules were placed in basket in each dissolution vessel to prevent floating. Ten ml of the samples was withdrawn from dissolution media at suitable intervals and same amount was replaced with fresh buffer. The absorbance was measured at 238.5nm. Data obtained was subjected to kinetic treatment to understand release mechanism. Several mechanisms of drug release, such as zero order, first order, Higuchi, Korsmeyer Peppas and Hixon Crowell were studied to ascertain the kinetic modelling of drug release.

RESULT AND DISCUSSION

Some physical parameters of both formalin treated and untreated capsule shells were carried out. According to the physical parameters in Table 2 the length of the body of the capsule was found to be 17.9 and 18.3 mm for untreated and treated capsules, respectively. The external diameter was found to be 6.9 and 7.1 mm respectively for untreated and treated capsules. The formaldehyde treated capsules were found to be slightly thicker; as a result they displayed much better sustained release activity compared to an untreated capsule. The increase of thickness was possibly due to the formaldehyde treatment process. Average weight of empty capsules was found in the range of 93.8-94.9 mg. Treatment did not influence the average capsule weight significantly. During the dissolution of untreated capsule it was seen that the body and cap dissolved between 15 min. On the other hand during the dissolution of treated capsule it was been seen that the cap dissolved within 15 min but the treated body remained intact. Drug and polymer did not have any chemical or physical interaction was proved by FTIR studies.[15,16]

Table 2: Physical characteristics of empty gelatin capsules with or without treatment

Type of Capsules	Length (mm)		External Diameter (mm)		Thickness (mm)		Weight of empty capsule (mg)
	Cap	Body	Cap	Body	Cap	Body	
Untreated	10.9 ± 0.1	17.9 ± 0.4	7.4 ± 0.2	6.9 ± 0.1	0.2 ±0.1	0.1 ± 0.0	93.8 ± 2.2
Formalin Treated	-	18.3 ±0.1	-	7.1 ±0.1	-	0.1 ±0.0	94.9 ± 2.1

From the zero order release kinetics, it was found that the formulations using 50 mg polymer (in each polymer plug) gave more than 93% release in 10 hr. The highest percent release was found for F-4 (HPMC K100LV) which was 98.77%. The formulations using 75mg polymer did not show any significant change in releasing drug. It also gave more than 90% release in 10 hr. The highest release was for F 8 (HPMC 50 cP) which was 96.13%. The percent release of F-9 (HPMC 100 cP) and F-10 (Na-CMC) were 96.46% and 94.81%, respectively. But in case of the formulations using 100 mg polymer, a very significant change was observed in releasing drug from the formulations. The highest release was for F 15 (HPMC 50 cP) which was 85.15%

whereas F-16 (HPMC 100 cP) and F-17 (Na-CMC) showed 79.49% and 83.05% release, respectively. In case of F-18 (HPMC K100LV), F-19 (carbopol-971), F-20 (methocel K15), F-21 (xanthan gum) the percent releases were 82.79%, 79.79%, 76.78%, 83.41%, respectively.

Here, it was found that by increasing the amount of polymer, release rate was decreased. The result was similar with the findings of some previous studies.[17-21] The graphs of zero order release kinetics gave a very clear concept that the release rate was above 90% when we used 50 mg and 75 mg polymer but in case of using 100 mg polymer the release rate was 70% to 85% in 10 hr (Figure 1). That means, in 12 hr these formulations can give a satisfactory result which is the most desire in pulsatile drug delivery system.

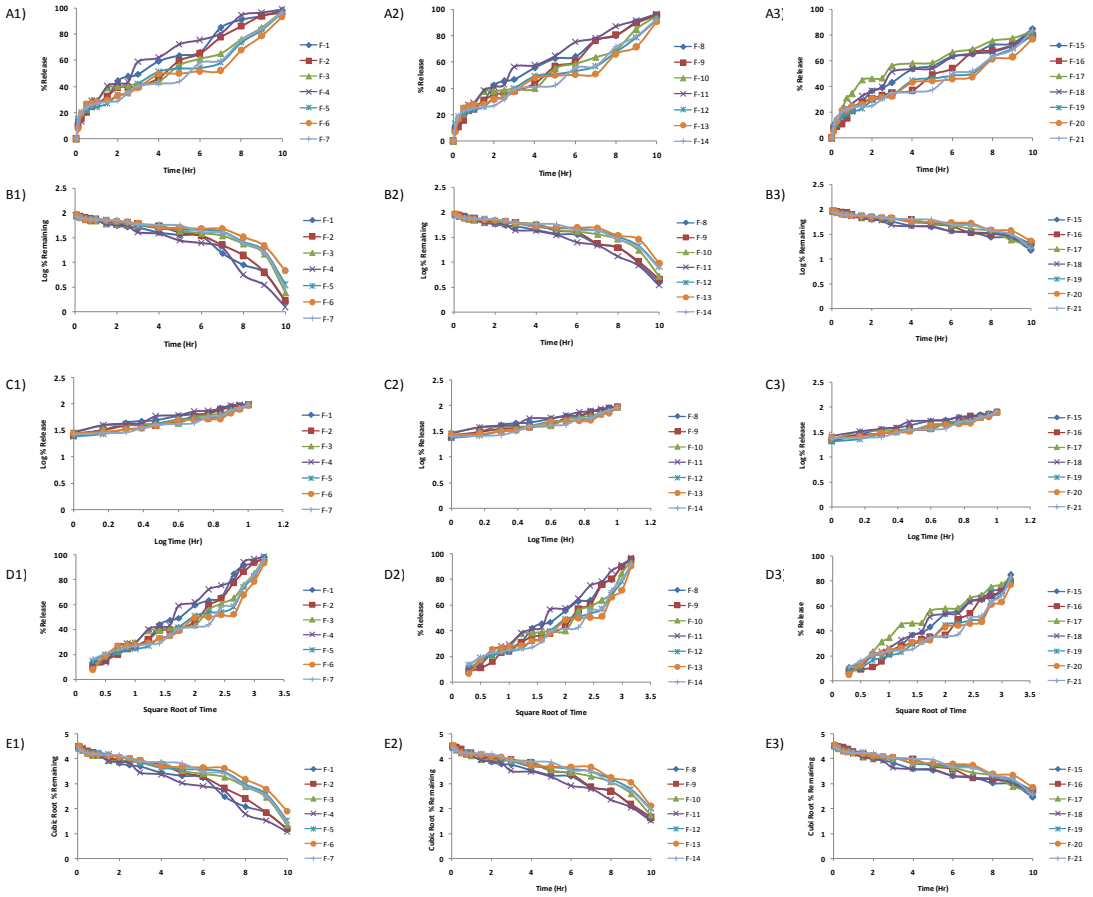

Figure 1. Zero order release kinetics (A), first order release kinetics (B), Korsmeyer Peppas release kinetics (C), Higuchi Kinetics (D) and Hixson-Crowell release kinetics (E) of formulation F1-F7, F8-F14 and F15-F21 containing different polymer grades used in the study with an amount of 50 mg (1), 75 mg (2) and 100 mg (3), respectively.

It was observed from Table 3 that most of the formulations followed Higuchi kinetic equation and zero order release kinetics. Few formulations also followed Korsmeyer- Peppas equation. The *in vitro* data was also subjected to Hixon-Crowell model. The 'n' values from the Korsmeyer Peppas plots concluded that all the formulations followed Super case II transport.[22] Case II generally refers to erosion of polymeric chain and anomalous transport (non-fickian) which refers to a combination of both diffusion and erosion controlled drug release.

Table 3. Interpretation of release rate constants and R^2 values for different release kinetics

Formulations	Zero Order		First Order		Korsmeyer		Higuchi		Hixson Crowell	
	R^2	K_0	R^2	K_1	R^2	n	R^2	K_H	R^2	K_{HC}
F-1	0.953	8.864	0.885	-0.139	**0.983**	1.44	0.981	30.46	0.963	-0.294
F-2	**0.972**	8.793	0.841	-0.132	0.966	1.385	0.967	30.09	0.940	-0.284
F-3	0.939	7.638	0.746	-0.101	0.914	1.45	**0.946**	25.74	0.847	-0.227
F-4	0.937	9.121	0.897	-0.157	0.979	1.479	**0.987**	31.55	0.970	-0.315
F-5	0.953	7.549	0.751	-0.092	**0.962**	1.346	0.926	25.21	0.868	-0.216
F-6	**0.917**	6.922	0.765	-0.074	0.915	1.381	0.906	23.14	0.852	-0.184
F-7	**0.950**	7.523	0.732	-0.096	0.920	1.343	0.893	24.74	0.851	-0.221
F-8	0.955	8.402	0.894	-0.107	0.986	1.426	**0.987**	28.95	0.962	-0.249
F-9	**0.975**	8.678	0.891	-0.106	0.966	1.351	0.967	29.88	0.955	-0.251
F-10	**0.938**	7.490	0.802	-0.085	0.891	1.439	**0.938**	25.30	0.893	-0.206
F-11	0.939	8.664	0.932	-0.117	0.985	1.469	**0.992**	30.09	0.981	-0.266
F-12	0.955	7.176	0.824	-0.073	**0.965**	1.335	0.936	24.06	0.902	-0.187
F-13	**0.915**	6.663	0.783	-0.064	**0.915**	1.371	0.914	22.42	0.861	-0.167
F-14	**0.955**	7.254	0.817	-0.076	0.927	1.310	0.903	24.02	0.938	-0.160
F-15	0.943	7.288	0.968	-0.066	**0.991**	1.381	0.990	25.19	0.981	-0.177
F-16	0.967	7.298	0.977	-0.060	0.968	1.310	0.977	25.34	**0.985**	-0.169
F-17	0.846	6.890	0.934	-0.058	0.936	1.356	**0.968**	24.11	0.958	-0.16
F-18	0.903	6.969	0.958	-0.060	0.975	1.436	**0.984**	24.39	0.959	-0.164
F-19	0.960	6.660	0.933	-0.053	**0.972**	1.280	0.963	22.88	0.958	-0.151
F-20	0.922	5.944	0.910	-0.045	0.934	1.339	**0.945**	20.26	0.932	-0.130
F-21	**0.949**	6.389	0.868	-0.054	0.915	1.285	0.907	21.21	0.914	-0.149

CONCLUSION

In the present study, a pulsatile release system for amlodipine besylate with appropriate amounts of excipients is developed on the lines of novel drug delivery systems. Universally sustained and controlled-release products provide a desired therapeutic effect, but fall for diseases following biological rhythms. There is a constant need for new delivery systems that can provide increased therapeutic benefits to the patients. Pulsatile drug delivery system is one such system that, by delivering drug at the right time, right place and in right amounts, holds good promises of benefit to the patients suffering from chronic problems like arthritis, asthma, hypertension etc. In this work, the results suggested that lag time is dependent on the nature of the materials employed in plug preparation and found that position of the plug in the capsule body significantly affects lag time. It is evident that an increase in the filler concentration in the plug results in an increase in lag time. Finally, it is possible to release a drug over a predetermined period of time with specific release rates by manipulating the amount of polymers used to prepare plugs. So it can be concluded that the prepared pulsatile drug delivery

system of amlodipine besylate capsule can be considered as one of the promising formulation technique for chronotherapeutic management of hypertension. Although this type of drug delivery technology enables the incorporation of drug molecules into a new delivery system, thus providing numerous therapeutic and commercial advantages, it should be pointed that these drug delivery systems are still in the early developmental stage.

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DISCLOSURE

The authors have no conflict of interest.

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