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Design and Development of Dual-drug Loaded Pulsatile Capsule for Treatment of Hypertension- *In Vitro* and *Ex Vivo* studies

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Abstract

The frequency of incidence of cardiovascular events in hypertensive patients is higher in the early morning hours between 5 a.m. to noon due to sharp increase in Blood pressure (BP), thereby suggesting a circadian pattern. Currently available antihypertensive medicines for bed time or morning dosing cannot address this early morning surge in BP. The aim of the present study was to design and evaluate a pulsatile dosage form to program the release of dual antihypertensive drugs to mimic the circadian pattern of BP. The optimized formulation meant for bedtime dosing, consisted of an insoluble capsule body housing a layer of swellable polymer, a Telmisartan (TELMI) tablet sealed by an erodible polymer tablet. The capsule body was closed with water soluble cap containing fast releasing Amlodipine (AMLO)-xylitol granules. The developed formulation was studied for physical characteristics, lag time determination, *in vitro* release, and *ex vivo* dissolution absorption. The capsule cap dissolved in acidic pH to release 98.67% of AMLO within 3 h. The swellable polymer layer at the base of capsule pushed the plug along with TELMI tablet out after a lag time of 6-7 h with 77.97% drug being released at the end of 12 h providing a time controlled need based release. *In vitro-ex vivo* studies revealed better degree of correlation in pulsatile capsule compared to marketed one. Thus this approach can be useful for timed release of combination antihypertensive medication and may provide effective 24-h control of BP in hypertensive patients.

Keywords: BP, circadian, chronotherapy, dual drug, Amlodipine, Telmisartan

1.0 Introduction

Large amount of scientific literature over the past decade documented a noteworthy predictable trend in the variability of biological processes and functions with circadian clock[1]. A host of diseases and conditions like hypertension, diabetes, asthma, arthritis, gastric ulcers, hypercholesteremia etc. are now widely reported to follow a biological rhythm. Hypertension is a disease condition which is a major whistleblower for existing cardiovascular conditions. Blood Pressure has now been established to exhibit 24 h variation with a peak in the morning. Elevated morning levels of various hormones like plasma norepinephrine and plasma renin can trigger marked coronary vasoconstriction which results in increased peripheral resistance during early morning hours and is lowest at night[2-4]. Studies indicate that the rate of rise of Blood pressure (BP) coincides with the diurnal activity and could be an important risk predictor of morning stroke, myocardial infarction or acute coronary conditions[5-9]. Thus cardiovascular events have been established to display a prominent 24-hour variation, with a significant morning peak both in hypertensive and normotensive subjects[10]. The currently available antihypertensive therapies deliver the drug as immediate release or controlled release and thus provide sub optimal doses during the time of maximum need.

Such cases require unique drug delivery technology which would ensure that peak and trough concentration of antihypertensive medication is in sync with the systolic and diastolic BP respectively[11]. This synchronization of biological rhythms with medical treatment is called chronotherapy [12-15]. Several approaches to chronotherapeutic drug delivery exist, of which pulsatile drug delivery systems have been developed to closely mimic emerging chronotherapeutic views [16]. Such systems comprise of a drug reservoir, surrounded by a barrier which either erodes, dissolves, or ruptures. For matrix type eroding tablet systems, a potential problem is the retardation provided by the polymer matrix which prevents

immediate drug release after barrier loss or may cause a premature release, particularly observed with highly water-soluble drugs. Capsular systems like the Pulsincap™ system which consists of an insoluble capsule body and a swellable plug overcomes this disadvantage, being independent of the nature of the content. Studies carried out in the past have used non-approved plug material in these systems. This problem can be overcome with devices of similar shape comprising of insoluble capsule shells and swellable or degradable plugs made of approved substances, such as hydrophilic polymers or lipids [17-20].Currently there are very few formulations available such as calcium channel blockers controlled-onset, extended-release (COER Covera-HS®)-verapamil10, CODAS-verapamil (Verelan PM®), graded-release long acting diltiazem (Cardizem LA®) tablets and the β-antagonist propranolol (InnoPran XL®), approved by FDA for chronotherapy of hypertension [11].

Previously, Nayak *et al.,* 2009[20] developed pulsatile capsule dosage form of valsartan, which can provide timed release of valsartan and may be helpful for patients suffering from morning surge. Similarly, Li *et al.,* 2007[21], and Zhou *et al.,* 2015[22] successfully developed a novel two-step release system by combining an effervescent osmotic pump tablet and pulsed-release tablet into one hard capsule, which exhibited drug-release that is in accordance with the circadian rhythms of cardiovascular disease. These pulsatile capsule designs have inspired us to re-design and formulate a programmed, dual drug loaded pulsatile release capsule for hypertension, which could mimic the circadian rhythm of BP and counteract its early morning rise, without causing a precipitous decline at night.

The pulsatile release technology has been widely studied in monotherapy for hypertension. A very commonly used drug combination in treatment of hypertension, Amlodipine (AMLO) and Telmisartan (TELMI) were selected as the combination antihypertensive drug therapy to compare it with the existing conventional marketed combination available. AMLO,(R,S)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-

methyl-1,4 dihydropyridine, is a calcium channel blocker[23]. TELMI, 2-(4-{[4-Methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl} phenyl)benzoic acid is a selective angiotensin-II (AT1) receptor antagonist [24]. Currently there is growing evidence to support the rationale of using a combination of renin–angiotensin system inhibitors along with a calcium channel blocker as an effective antihypertensive therapy. The TELMI-AMLO combination has demonstrated significantly greater BP reductions compared to each monotherapy and well documented safety profiles similar to placebo in patients with moderate to severe hypertension and high-risk patients[25]. Till date pulsatile systems containing dual drugs have not been studied. The two drugs used for the present study have been chosen such that Amlodipine exhibits a t_{max} of 6-7 h and Telmisartan has a t_{max} of 0.5-1.0 h. Amlodipine granules were released immediately after capsule cap dissolution while Telmisartan tablet was released after a lag time of 6-7 h. Thus through the designed night time dosed pulsatile formulation the two drugs should reach peak plasma concentrations at almost the same time around early morning which is the peak time for maximum cardiovascular events to occur.

2.0 Materials and methods

2.1 Materials

AMLO and TELMI was a gift sample obtained from Unichem Laboratories Ltd., Mumbai. Hard gelatin capsule shells (Size 0) were a gift sample from Dolphin® (Code-1269). Xylitol was purchased from Sigma Aldrich, USA. Guar gum (GG), xanthan gum (XG), hydroxypropylmethyl cellulose (K4M), hydroxypropylmethyl cellulose (K100M), sodium alginate (SA), microcrystalline cellulose (PH101), potassium permanganate, magnesium stearate and talc was purchased from Loba Chemicals, Mumbai. Directly compressible

lactose (DCL), anhydrous formaldehyde were purchased from Merck, Mumbai. All other excipients and chemicals used were of analytical grade.

2.2 Preparation of pulsatile Capsules

The pulsatile capsules were prepared by assembling AMLO granules, erodible tablet, TELMI core tablet and swelling polymer bed into a water insoluble capsule body. The preparation methods of granules, core tablet, water insoluble capsule body and erodible tablet are given below. The assembly is shown in Fig. 1:

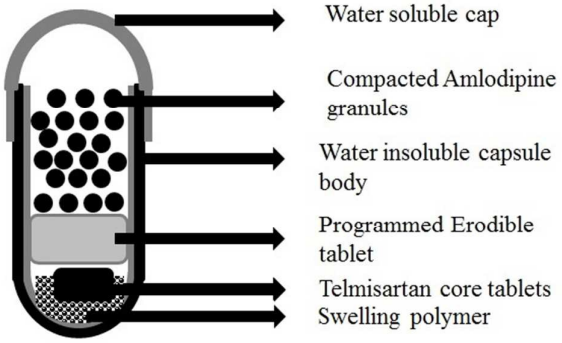

Fig. 1: Assembly of the pulsatile capsule

2.2.1 Preparation of AMLO granules

AMLO granules were prepared with xylitol by melt granulation technique. Required amounts of xylitol was melted in a china dish to which AMLO was added, stirred rapidly, cooled and passed through #22 sieve to obtain granules in 1:4 (G1), 1:5 (G2), 1:6 (G3) ratios.

2.2.2 Preparation of TELMI core tablets

TELMI core tablets were prepared by direct compression method using a superdisintegrant. Four formulations (C1-C4) were prepared by varying the ingredients. Each ingredient was sifted through mesh No-60. The mixture was blended uniformly. After sufficient mixing of drug with the other tablet components, the tablets were directly compressed using a 6.5 mm punch. The formulation chart is given in Table 1.

Table 1: Table 1: Formulation chart of TELMI core tablets

Ingredients	C1	C2	C3	C4
Telmisartan	40	40	40	40
Avicel PH101	52	50	48	46
PVP K30	4	4	4	4
Croscarmellose sodium	-	2	4	6
Mag. Stearate	2	2	2	2
Talc	2	2	2	2

2.2.3 Preparation of water insoluble capsule body

Water insoluble capsule body was prepared by cross-linking of gelatin capsules with formaldehyde[26, 27]. Briefly, 25 mL of 37% w/v formaldehyde was taken into a desiccator, to which a small amount of potassium permanganate was added to generate formalin vapours. Wire mesh containing empty hard gelatin capsule bodies were exposed to formalin vapours. Vacuum desiccator was closed tightly, allowing reaction to proceed for 6 h. Capsule bodies were dried at 40 ºC for 12 h to ensure completion of reaction between gelatin and formalin vapours. Capsule bodies were removed and dried at room temperature to facilitate removal of residual formaldehyde. The capsule bodies were capped with untreated caps and stored in sealed polythene bags.

2.2.4 Preparation of erodible tablets

Studies carried out by Nayak *et al.,* 2009[20] reported the use of low cost, shear stable, non-toxic and bio-degradable hydrophilic polymers for erodible tablet plugs in pulsatile release systems to replace non approved plug materials. The erodible tablets in the current study were prepared using combination of DCL with GG and XG by direct compression method as reported by Nayak *et al*., 2009. The formulation chart for the erodible tablets is listed in Table 2.

Table 2: Formulation chart for erodible tablets

Formulations	DCL	GG/XG	Magnesium stearate	Talc	Total wt. of tablet
E1	46	50	2	2	100
E2	21	75	2	2	100
E3	-	96	2	2	100
E4	69	75	3	3	150
E5	31.5	112.5	3	3	150
E6	-	144	3	3	150

2.3 Characterization & Evaluation

The following characterization and evaluations were carried out in triplicate (n=3).

2.3.1 Compatibility studies

Fourier transform infrared (FT-IR) spectrometry and thermal analysis (DSC) were carried out to study the compatibility between drug and polymers used. Fourier transform infrared (FT-IR) spectrum was obtained by KBr pressed pellet technique using a Shimadzu, FT-IR 8400S. Differential scanning calorimetry (DSC) was carried out using a Shimadzu, DSC 60.

2.3.2 Evaluation of prepared granules

2.3.2.1 Micromeritic properties of prepared granules

The prepared granules were studied for different micromeritic properties such as bulk density (ρ_b), tapped density (ρ_t), compressibility index, Hausner ratio and angle of repose (θ). The following studies were carried out with a minimal quantity of 10 g and performed according to procedures described and defined in Indian Pharmacopeia.

Bulk density (ρ_b) is the ratio of total mass (M) of granules to the bulk volume (V_b) of granules. Tapped density (ρ_t) is the ratio of the mass (M) of the powder to the final tapped volume (V_t). Accurately weighed quantity of granules was carefully placed into the graduated cylinder through a funnel, and was tapped until there was no further reduction in volume.

Compressibility index and the Hausner ratio were determined by measuring both the bulk volume and the tapped volume of a powder. The compressibility index (C) and Hausner ratio (H) may be calculated using measured values for bulk density (ρ_b) and tapped density (ρ_t) as following (equation 1 and 2):

$$\%\mathbf{C} = \frac{\rho\mathbf{t}-\rho\mathbf{b}}{\rho\mathbf{t}}\mathbf{X100} \quad \text{.....1}$$

$$\mathbf{H} = \rho\mathbf{t}/\rho\mathbf{b} \quad \text{.....2}$$

Angle of repose is the maximum angle possible between the surface of a pile of the granules/powder and the horizontal plane. Fixed funnel method was employed. The angle of repose (θ) was calculated using the given formula (equation 3):

tan (θ) = Height/Radius…….3

2.3.2.2 Drug content of granules

The drug content of AMLO granules was estimated using UV-Visible Spectrophotometer, Shimadzu-1800. An accurately weighed quantity of granules (equivalent to 10 mg of AMLO) was taken and dissolved in 10 mL of methanol; from this 1 mL of solution was diluted to 10 ml and assayed for drug content at 366 nm. The samples were filtered prior to analysis.

2.3.2.3 *In vitro* release studies of granules

Drug release studies from formulations were carried out in triplicate, employing Electrolab dissolution tester-USP XXII, basket type, TDT-08L, using 900 mL of pH 1.2 Hydrochloric acid buffer as dissolution medium at 75 rpm and 37 ^{o}C ± 0.5 ^{o}C for 1 h. An aliquot of sample was withdrawn periodically at every 10 min interval and the volume was replaced with an equivalent volume of plain dissolution medium. Samples were analyzed spectrophotometrically at 366 nm after suitable dilution.

2.3.3 Evaluation of TELMI core tablets

2.3.3.1 Pre-compression Parameters

The tablet blends were evaluated for their bulk density, tapped density, Carr's index and other flow properties as explained above (section 2.3.2.1).

2.3.3.2 Post-compression Parameters

The post compression parameters such as tablets hardness, weight variation, friability, drug content and disintegration time were studied. All the studies were carried out by using standard procedures listed in Indian Pharmacopeia, 1996.

2.3.3.3 *In vitro* release studies of tablets

In vitro drug release studies were carried out using 900 mL of pH 7.4 phosphate buffer as dissolution medium. The procedure employed is the same as the method described in "*In vitro* release studies of granules". Samples were analyzed spectrophotometrically at 296 nm after suitable dilution.

2.3.4 Evaluation of insoluble capsule bodies

The prepared formalin treated capsule bodies were evaluated for physical tests such as visual defects dimension changes, solubility and quantitative chemical test for free formaldehyde.

2.3.4.1 Quantitative chemical test for free formaldehyde

Twenty five formaldehyde treated capsule bodies were cut into small pieces and transferred into a beaker containing 20 mL distilled water and stirred for 1 h. The solution was filtered and washed and made up to 50 mL. From the above solution 1 mL was transferred into a mixture of 4 mL water and 5 mL acetyl acetone reagent. The prepared solution was warmed

to 40 ºC for 40 min. The resulting solution was compared with colour intensity of standard solution (0.002 w/v formaldehyde solution) and should be less than standard[27].

2.3.5 Evaluation of erodible tablet

2.3.5.1 Pre and Post Compression Parameters

The prepared erodible tablets were evaluated for pre and post compression parameters. The procedure employed is same as described before in section (2.3.3.1 & 2.3.3.2).

2.3.5.2 Water uptake studies

The water uptake studies were carried out by initially exposing erodible tablet to pH 1.2 hydrochloric acid (HCl) buffer for 3 h followed by pH 7.4 phosphate buffer for the next 9 h (37±2 ºC, 75 rpm). At predetermined time points, tablets were carefully blotted with tissue paper to remove the surface water and then weighed (n=3). Water uptake was calculated by using following equation 4.

Water uptake (%) = (Wet Wt. – Dry Wt.)*100/Wet Wt……4

2.3.6 Lag time determination

The lag time was determined by visual observation of the pulsatile capsules in a USP type II apparatus (Medium: 0.1 N HCl for 3 h followed by phosphate buffer, pH 7.4; 37±0.5 ºC; rotation speed: 75 rpm). The time at which the plug was pushed out and drug release started was considered as the lag time[20].

2.3.7 *In vitro* release and kinetics of chronotherapeutically active pulsatile capsular drug delivery system

In vitro drug release studies from the assembled capsules were carried out in triplicate, employing Electrolab dissolution tester-USP XXII, basket type, TDT-08L, initially using

900 mL of pH 1.2 HCl buffer for 3 h followed by pH 7.4 phosphate buffer as dissolution medium for 9 h at 75 rpm and 37 ºC ± 0.5 ºC. An aliquot of sample was withdrawn periodically at every 0.5 h interval and the volume was replaced with an equivalent volume of plain dissolution medium. Samples were analyzed for presence of AMLO and TELMI, spectrophotometrically at 296 and 366 nm respectively after suitable dilution.

The *In vitro* release studies data were integrated into various mathematical models to determine the best-fit release profile model using PCP.Disso-V2.08 software.

2.3.8 Continuous dissolution-absorption study using everted intestine segment (*Ex vivo* studies)

The system consisted of USP Type II dissolution apparatus and a side-by-side perfusion apparatus holding isolated everted intestine segment[28]. In this system, drug dissolution from the delayed release capsule and permeation across everted intestine occurred simultaneously (n=3). The perfusion apparatus consisted of two glass tubes A and B, connected together as shown in Fig. 2. Tube B had a bent cannula at its lower end, and tube A, a straight cannula at its lower end. The distance between the cannulas was kept constant. The isolated everted rat intestinal segment was fixed between the ends of the tubes A and B. The ends of the intestine were tied in position with a thread and contained 20 mL of KR solution. The apparatus was immersed completely into the dissolution vessel. Initially dissolution study of marketed formulation and prepared chronotherapeutic capsules were carried out in 0.1 N HCl for 3 h in separate setup after which they were removed and transferred to phosphate buffer pH 7.4. Simultaneously continuous dissolution-absorption study was carried out for samples at 37±0.5 ^{o}C. The samples were withdrawn from the dissolution flask (5 mL) as well as from cannula (3 mL) at predetermined time intervals and replaced with fresh media. The samples

were analyzed at 366 and 296 nm using UV-Visible spectrophotometer. The setup is shown in supplementary data (Supplementary Fig.1).

3.0 Results and Discussion

AMLO, a sparingly soluble calcium channel receptor blocker was formulated as a solid dispersion with a sugar (xylitol) by melt granulation technique. TELMI, a potent angiotensin II inhibitor with its poor solubility was mixed with Avicel PH101 and PVP K30 as binders and was formulated into mini tablets by direct compression technique.

Characterization and evaluation of the AMLO granules and TELMI tablets were carried out along with drug release studies of the assembled capsules. The effect of various parameters such as type and weight of swellable polymer, type of hydrophilic polymers used in erodible tablet, weight of erodible tablet were investigated in order to determine the lag time and drug release profiles. Formalin treated water insoluble capsule body were prepared and used to house AMLO granules, erodible tablet, TELMI core tablet and swelling polymer bed.

3.1 Compatibility Studies

The spectral peaks and DSC thermograms of AMLO pure drug, AMLO-xylitol granules, pure TELMI and TELMI powder blends were sketched, compared and enclosed in the supplementary data (Supplementary Fig. 2, 3, 4 and 5 respectively).

Pure AMLO showed IR absorption bands at 3157.50 cm^{-1} for the stretching vibration of N–H bond in the dihydropyridine ring; 1301.99 cm^{-1} for the ethyl ester; 1182.40 cm^{-1} for sulphonic acid salts and 1126.47 cm^{-1} for the aliphatic esters. These characteristic IR absorption bands of AMLO were all retained in the granules indicating no interaction between the drug and granule excipients.

IR spectra of TELMI showed a characteristic peak at 3059.20 cm^{-1} for Aromatic –CH stretch; 2945.90 cm^{-1} for Aliphatic -CH stretch; 1697.41 cm^{-1} for –COOH; 1614.47 cm^{-1} for

Aromatic C=C bending and stretching; 1458.23 cm^{-1} for C-H bending and 1381.80 cm^{-1} for –OH bending and –C=O stretching vibrations. Characteristic peaks of TELMI seemed to be preserved in physical mixture which proved that there was no chemical interaction between telmisartan and tablet components.

The DSC thermograms showed sharp endothermic transitions corresponding to the melting points of the drugs and the excipients, which indicated that the drugs did not chemically interact with the components of the formulations.

3.2 Evaluation of Amlodipine-Xylitol granules

3.2.1 Micromeritic properties of Amlodipine-Xylitol granules

The flow properties of the prepared granules were studied by calculating the angle of repose (θ), % compressibility index (CI) and Hausner's ratio (HR). The study results are presented in supplementary data sheet. The values of θ ranged from 24.1±0.25 to 26.7±0.23, values of CI were found to be in the range of 14.91±0.13 to 15.91±0.23 % and HR was in the range of 1.175±0.16 to 1.189±0.14. The standard ranges for θ, CI and HR to indicate good flow were 25-30, 5-15% and <1.25 respectively. The observed results were comparable with the standards which indicated that the prepared granules possessed reasonably good flow potential. The values of bulk density ranged between 0.490±0.39 to 0.508±0.32 g/mL and tapped density ranged between 0.583±0.32 to 0.598±0.27 g/mL. Density differences between the formulations were negligible indicating that the prepared granules were non-aggregated and spherical in nature.

3.2.2 Drug content of AMLO-xylitol granules

The drug content of G1, G2 and G3 was found to be 98.52±0.31, 98.02±0.34 and 97.69±0.22 % respectively.

3.2.3 *In vitro* Drug release studies of AMLO-xylitol granules

The drug release studies for all the 3 batches of AMLO-xylitol granule formulations were carried out in pH 1.2 HCl buffer. Since, AMLO-xylitol granules where expected to be released immediately in stomach therefore, pH 1.2 HCl buffer solution was used for the study to mimic gastric environment. The release profile is recorded in Fig. 2. Percentage drug release at the end of 1 h was 97.25±2.29 for G1 formulation and decreased with increase in quantity of xylitol (95.91±2.35 for G2 and 86.23±2.33 for G3). Formulation G1 (1:4) showed good drug release and hence was selected as the optimized formulation in the pulsatile capsules as immediate release dose.

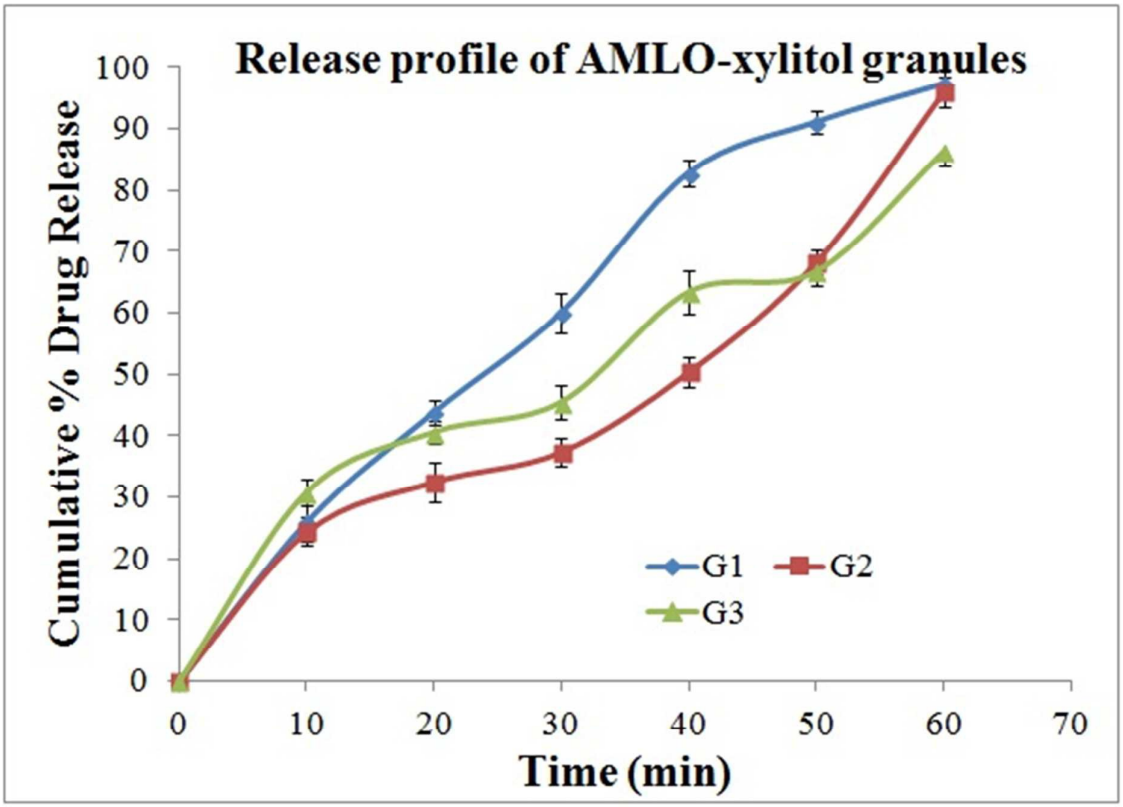

Fig. 2: Release profile of AMLO-xylitol granules. n=3

3.3 Evaluation of TELMI core tablets

3.3.1 Evaluation of Pre-compression properties of TELMI powder blend

The TELMI powder blends were studied for various micromeritic properties (results of each respective batch were reported in supplementary data sheet) and showed θ in the range of 30.04±0.28 to 32.01±0.23, CI in the range of 15.07±0.38 to 16.69±0.38 % and HR in the range of 1.177±0.28 to 1.20±0.28. The observed results were comparable with the standards which indicated that the prepared granules possessed reasonably good flow potential. The values of bulk density ranged between 0.509±0.21 to 0.524±0.25 g/mL and tapped density ranged between 0.611±0.25 to 0.618±0.24 g/mL. There was not much difference in densities observed which indicated no aggregation in the TELMI powder blend.

3.3.2 Evaluation of post compression parameters and *In vitro* drug release of prepared TELMI core tablets

The prepared TELMI tablet batches showed uniform thickness (3.51 mm) and diameter (6.64 mm). All the batches of tablets were studied for hardness, friability, weight variation and disintegration. The hardness was found to be between 2.21 to 2.31 kg/cm^2. Friability was between 0.50 to 0.54 % which was within official limits of 1.0% (Table 3). The results of drug content for C1, C2, C3 and C4 batches of TELMI formulations are listed in Table 3. The results indicated the drug content to be in the range of 97.85 to 99.05 %.

Table 3: Post-compression parameters of TELMI core tablets

Batch code	Weight Variation	Thickness (mm) Mean ± SD*	Hardness (kg/cm^2) Mean ± SD*	Friability (%) Mean ± SD*	Disintegr-ation time (min) Mean ± SD*	% Drug content Mean ± SD*
C1	Pass	3.51± 0.015	2.25± 0.23	0.53± 0.115	15±0.4	98.32± 0.25
C2	Pass	3.51± 0.01	2.27± 0.32	0.50± 0.15	10±0.5	98.98± 0.35
C3	Pass	3.51± 0.01	2.31± 0.27	0.51± 0.2	9±1.0	97.85± 0.29
C4	Pass	3.51± 0.011	2.21± 0.33	0.54± 0.14	4±0.5	99.05± 0.31

*Standard Deviation, n=3

The disintegration time was in the range of 4 to 15 min. The C1 tablet (without disintegrant) took more than 15 min to disintegrate and TELMI release was slow. Thus in order to get rapid drug release a superdisintegrant, croscarmellose sodium (CCS) was added to the formulation. Chemically CCS is a cross linked sodium carboxymethylcellulose having high swelling capacity with little tendency to gel. This internal cross linking reduces its water solubility while still allowing the material to swell and absorb almost 4-8 times its weight in water in less than 10 sec. Crosslinking allows enhanced bioavailability of drug through superior drug dissolution. The disintegrant particles possess low cohesiveness and hence enhance the porosity and "wicking" action which subsequently leads to rupture and disintegration of tablet[29].

With the addition of CCS the disintegration time drastically decreased in the subsequent formulations. The formulation C2 (2% CCS) disintegrated in 11-12 min, C3 (4% CCS) disintegrated in 8-10 min and C4 (6% CCS) disintegrated in 4-5 min. The *in vitro* drug release study was carried out in pH 7.4 phosphate buffer. The cumulative drug release profile is illustrated in Fig. 3. The formulation C4 was found to take minimum time to disintegrate and showed a maximum drug release of 78.19 % at the end of 1 h and was thus found suitable for use as the second pulse tablet in the delayed release capsule. TELMI core tablet was

expected to be released in intestine hence; pH 7.4 phosphate buffer was used for the study to mimic intestinal pH.

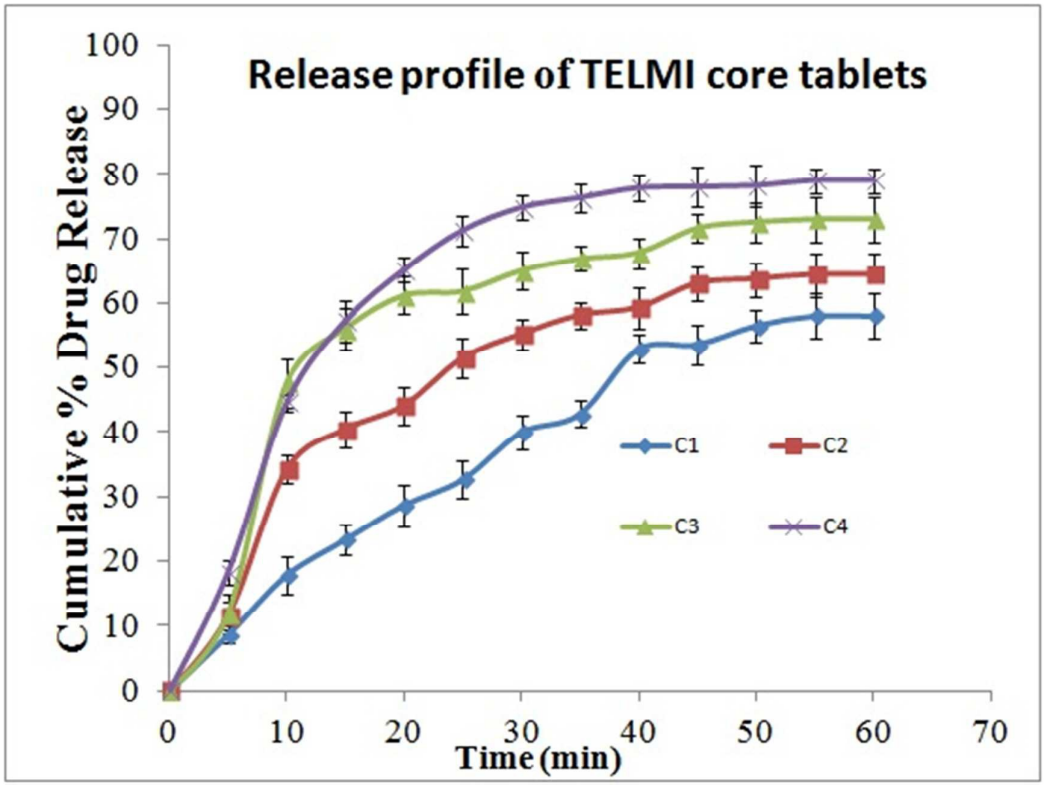

Fig. 3: Release profile of TELMI core tablets. n=3

3.4 Evaluation of Formalin treated capsule body

The formalin treated capsules were visually inspected for change in diameter, evaluated for solubility of capsule body and also for the presence of free formaldehyde. The change in diameter of capsule was not observed, whereas significant change in solubility was observed. It was observed that all normal capsules were dissolved within 15 min whereas, formalin treated capsules remained intact even after 8 h. When tested for free formaldehyde. The sample solution colour was less intense than the standard solution (0.002 w/v formaldehyde solution). Therefore, less than 20 µg free formaldehyde was reported per 25 test capsules. Formaldehyde is not on the ICH guideline lists for solvents and thus a control limit cannot be found. As per World Health Organization's guideline WHO/SDE/WSH/05.08/48,

formaldehyde is carcinogenic by inhalation but is not carcinogenic by the oral route. However, US Environmental Protection Agency's (guideline EPA 822-S-12-001) has established a maximum daily dose reference of 0.2 mg/kg per day for formaldehyde [30]. Therefore, the prepared formalin treated capsule body are safe for oral consumption.

3.5 Evaluation of erodible tablets

3.5.1 Micromeritic properties of erodible tablet blends

The polymer powder blends were studied for various micromeritic properties (results of each respective batch were reported in supplementary data sheet) and showed θ in the range of 24.05±1.92 to 28.45±1.69, CI in the range of 11.67±1.44 to 19.56±0.49 % and HR in the range of 1.122±0.02 to 1.278±0.015. As the amount of GG and XG were increased in the blend, the flow properties of the mixture decreased. The tablet batches showed uniform thickness (3.5 mm) and diameter (6.5 mm). The hardness of XG tablets were found to be 4.5 kg/cm^2, whereas the GG tablets showed hardness of 3.5 kg/cm^2. The weight variation and friability were within official limits of 7.5% and 1.0% respectively.

3.5.2 Water uptake studies

Few groups in the past have reported use of natural hydrophilic polymers in preparation of plug material, successfully replacing the non-approved ones for the pulsatile capsular systems[18, 20, 26, 27, 31, 32]. Hydrophilic polymers and gums have a tendency to swell in cold water forming viscous colloidal dispersions or sols by absorbing water. In the present study different amount of GG/XG and DCL were used to prepare erodible tablet/plug material.

Water uptake studies on the erodible tablets showed linear increase in weight of the tablets. The water uptake increased linearly as the amount of GG and XG increased. Fig. 4 compares the water uptake capacity of GG and XG erodible tablets with time. GG showed relatively more water uptake capacity and more viscosity than XG. Percentage water content of GG

tablets increased with time. But once the XG tablets absorbed sufficient water, it started eroding the polymer at the surface of the tablet (Fig. 5). Thus the GG was found to be a better polymer choice for preparing the erodible tablets in delaying the release of the core tablet from the capsule body. The pulsatile capsule was expected to move from stomach to intestine hence, water uptake studies of erodible tablet and evaluation of pulsatile capsule were carried out in pH 1.2 HCl buffer solution for 3 h, followed by phosphate buffer pH 7.4 for next 9 h to mimic the changes in GI pH.

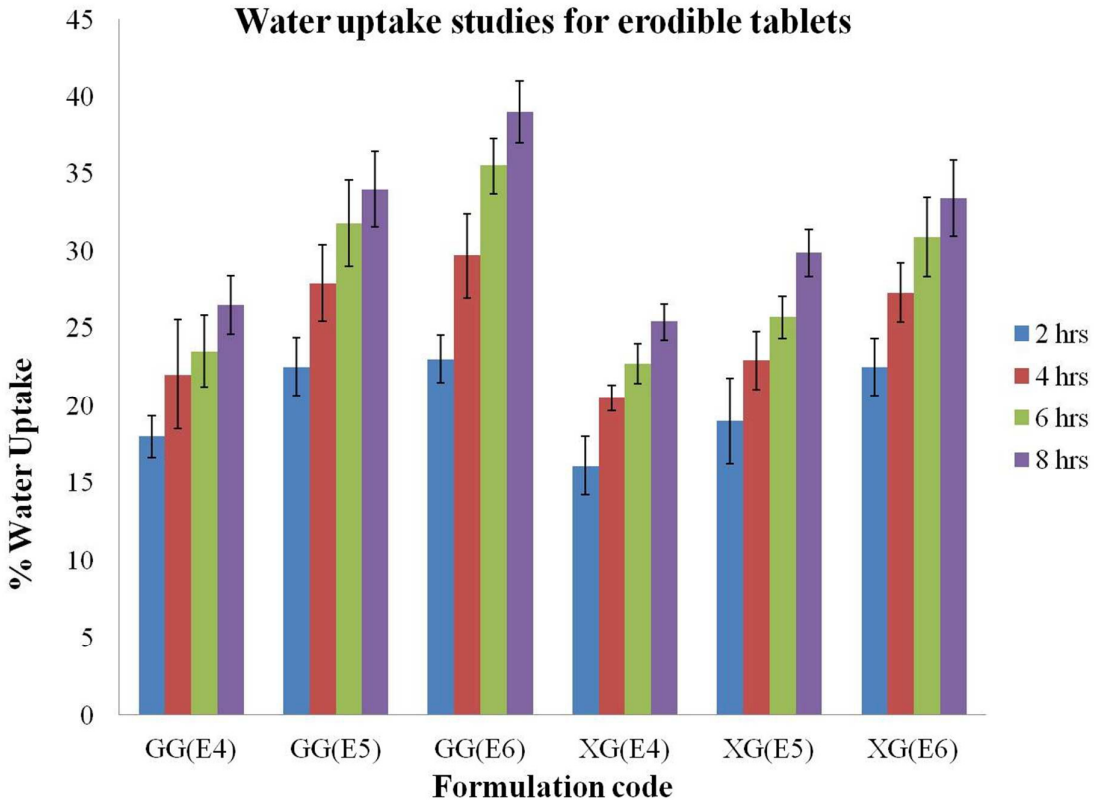

Fig. 4: Water uptake studies for erodible tablets of GG and XG. n=3

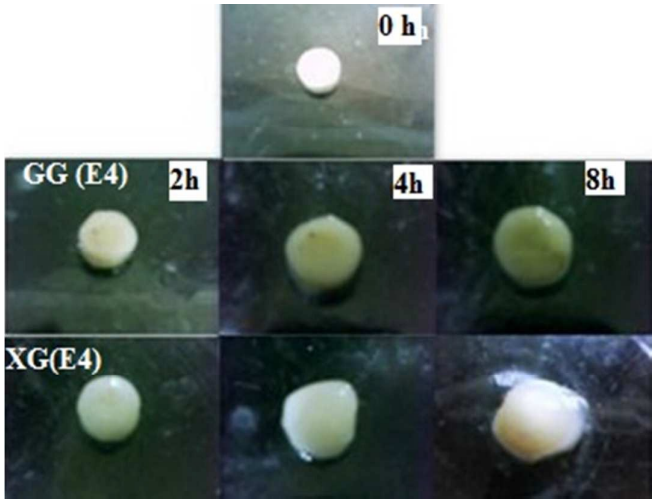

Fig. 5: Comparison of swelling of GG and XG erodible tablets

3.6 Pulsatile capsule

Pulsatile capsules containing AMLO-xylitol granules (G1) and second pulse of TELMI core tablet (C4) in the insoluble capsule body sandwiched between erodible polymer tablets and swellable polymeric layer were developed. When the capsule reaches the stomach fluids the cap dissolves and the AMLO granules are released. The swellable polymer layer at the base of the capsule body along with the erodible tablet swells up on absorbing body fluids and after a lag period of 6-8 h the plug is pushed out releasing the TELMI tablet. The mechanism is illustrated in Fig. 6. The weight variation of these pulsatile capsules were found to be within the official limits (<7.5%).

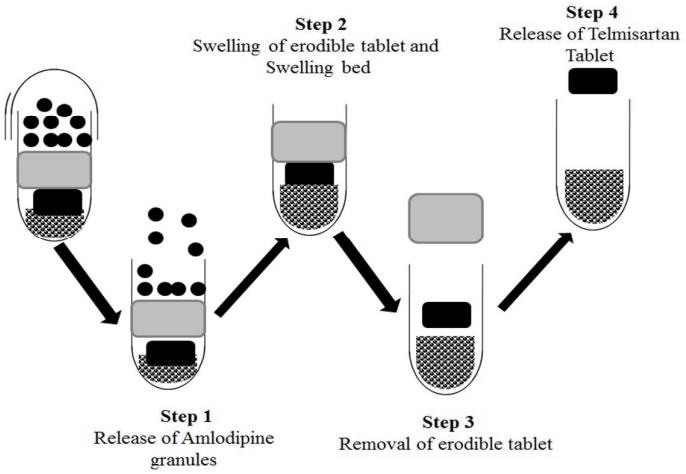

Fig. 6: Illusion of drug release mechanism of pulsatile capsule

3.6.1 *In vitro* drug release, kinetics & optimization of pulsatile capsule

Drug release studies were necessary to ensure that the erodible tablets could effectively seal the mouth of the capsule body. Thus dissolution studies of pulsatile capsules were carried out in 0.1 N HCl for 3 h, followed by phosphate buffer pH 7.4 for next 9 h.

Initially, 100 mg erodible tablets containing GG and XG were studied for drug release from pulsatile capsules containing 100 mg of swelling polymer (XG, GG and SA) at the base of the insoluble capsule body. But GG was not very effective as a swelling polymer as its swelling capacity was relatively lesser than XG/SA which was utilized to our advantage for use in erodible tablets. Hence further trials were carried out using different concentrations of GG in the erodible tablets and XG/SA as swelling polymer in the capsule. With the 100 mg erodible tablets, TELMI release started very fast, lag time was at 5±1 h and drug release was between 21.22 to 38.04% at the end of 12 h. In all the cases the erodible tablet remained at the mouth of the capsule and could not be expelled. Whatever drug was released was due to diffusion through the swollen erodible plug. Hence all the 100 mg erodible tablets proved ineffective to allow proper drug release after a desired lag time.

Next the swelling polymer at the base of the capsule was increased from 100 mg to 150 mg. Erodible tablets came out of the capsule faster. Complete drug release was seen by the end of 10 h. But lag time was around 4 h.

Subsequent trials were carried out using 150 mg erodible tablets of GG and XG with 150 mg of SA/XG as swelling polymer. Lag time for drug release was in the range of 6-7 h after which the plug was pushed out and rapid drug release was seen. Drug release was better from the GG plugged capsule bodies than the XG tablets. GG (E4-6) erodible tablets containing capsules with SA or XG swelling polymer were further compared for drug release. The pulsatile formulations were assembled containing first release AMLO granules and second release TELMI tablets plugged by 150 mg erodible tablets. These formulations were identified as F1-F3 containing E4, E5, E6 as 150 mg erodible tablets respectively and containing 150 mg SA/XG as swelling polymer. The drug release profiles for AMLO and TELMI from the capsule formulation containing GG erodible tablets are illustrated in Fig. 7. Based on the results of drug release studies F1 (SA) was selected as the optimized formulation as it showed a release of 98.67 and 77.97% of AMLO and TELMI by the end of 3.5 h and 12 h respectively (Fig.7).

Based on the data obtained from *in vitro* drug release studies, F1 (SA) was identified as the optimized formulation and was integrated into various mathematical models to determine the best-fit model to explain its drug release mechanism. F1 (SA) was composed of 5 mg of AMLO formulated as G1, 1:4 granules; 40 mg TELMI in 100 mg C4 mini tablets; 150 mg of E4 erodible tablet plug and 150 mg of SA as swellable polymer at the base of capsule body. The results indicated that, best-fit model (R^2=0.9563) for AMLO release from capsule formulation was Hixson-Crowell cube root law. The value of n determined from Hixson-Crowell equation was 0.5473 which followed anomalous transport mechanism for drug release. While that for TELMI release (R^2=0.9730) followed Power law. The value of 'n'

determined from Power law equation was greater than 1.0 which indicated that the drug release followed Non-Fickian mechanism, Super case II model (swelling controlled release).

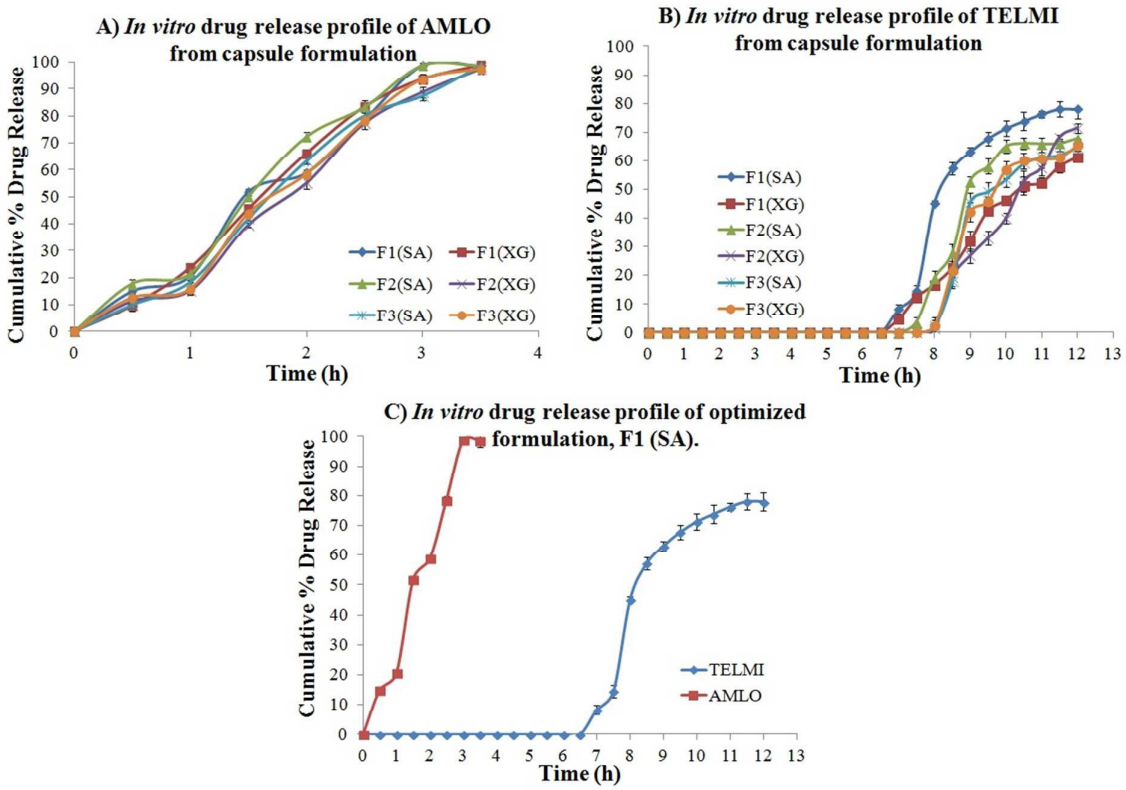

Fig. 7: *In vitro* drug release profile of A) AMLO from capsule formulation, B) TELMI from capsule formulation and C) optimized formulation, F1 (SA). n=3

3.6.2 Continuous dissolution-absorption studies (*Ex vivo* studies)

The *in vitro* dissolution and absorption studies of marketed tablets and F1 (SA) formulations were conducted. A fraction of the drug dissolved (Fd) and the fraction of drug absorbed (Fa) were calculated and plotted against time. The dissolution-absorption plots were constructed by plotting the Fa against Fd. From this the time for absorption, time for dissolution (mean dissolution time) and the time for intestinal permeation can be predicted.

AMLO exhibits a t_{max} of 6-7 h and TELMI 0.5-1.0 h. The optimized formulation, F1 (SA) dissolution was carried out initially in gastric media (0.1 N HCl) for 3.5 h followed by intestinal media (phosphate buffer pH 7.4) for 12 h. AMLO granules were released immediately after capsule cap dissolution while TELMI tablet was released after a lag time of

6-7 h. Thus through the designed pulsatile formulation the two drugs should reach peak plasma concentrations at almost the same time. In the case of marketed formulation, both the drugs were released immediately in gastric media (0.1 N HCl) which was available for absorption. The optimized formulation, F1 (SA) showed a better correlation between the fraction of drug dissolved and that absorbed which indicated better absorption and permeation of the dissolved drug. The results are illustrated in the Fig. 8 and 9.

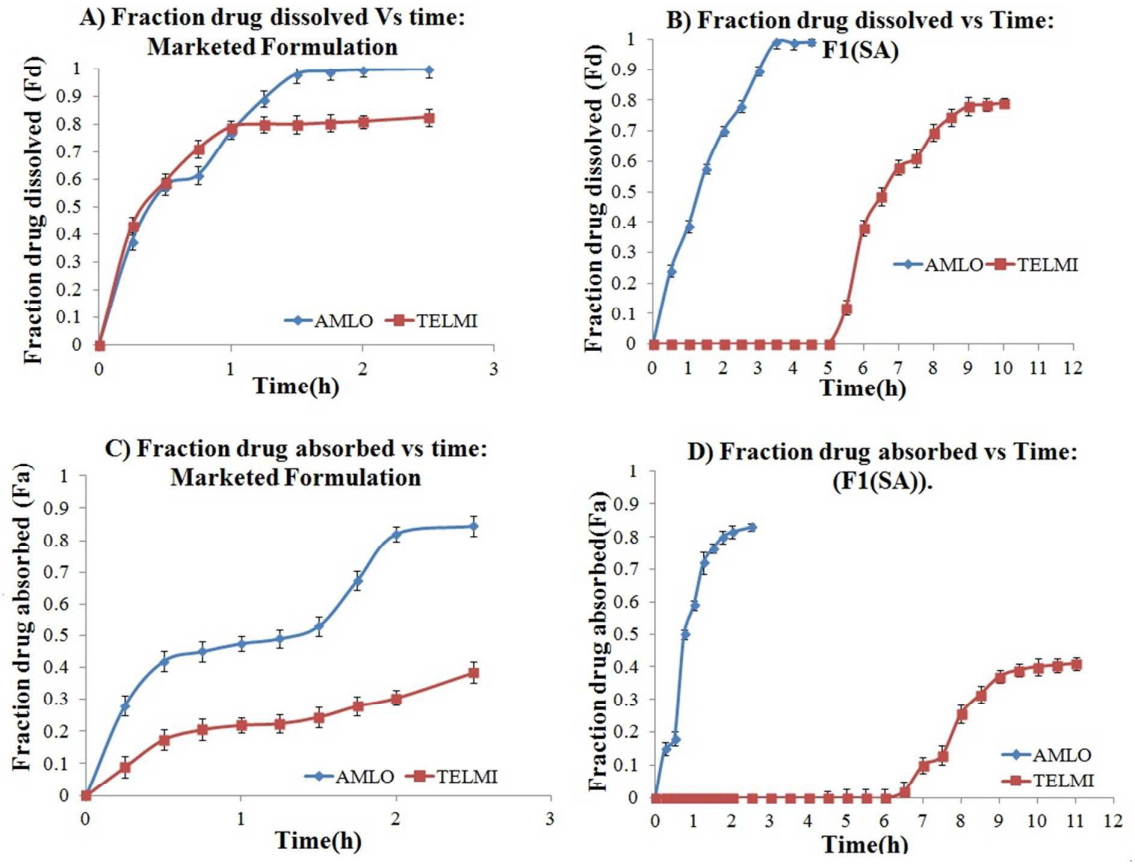

Fig. 8: Fraction drug dissolved Vs time ((A) Marketed and (B) F1(SA)), Fraction drug absorbed Vs time ((C) Marketed and (D) F1(SA)). n=3

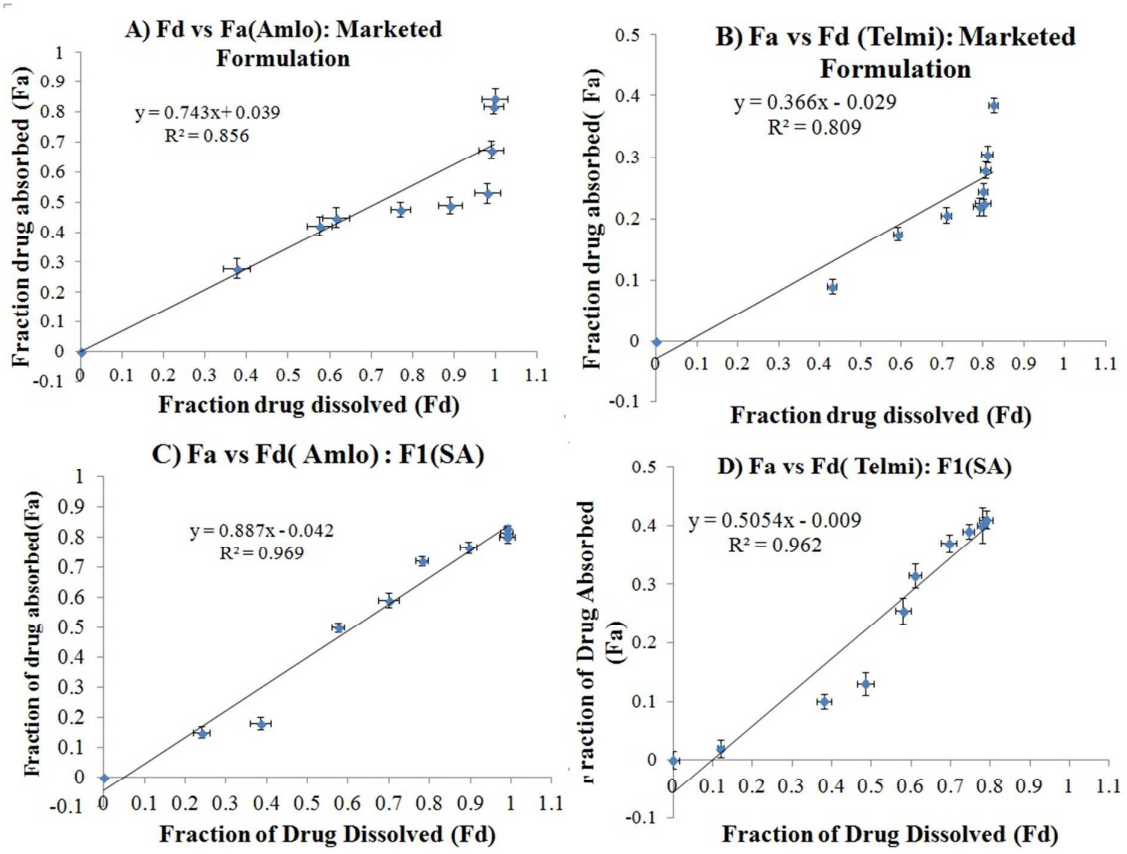

Fig. 9: Fraction drug dissolved Vs fraction drug absorbed ((A) Marketed AMLO, (B) F1 (SA), (C) Marketed TELMI and D) F1(SA). n=3

4.0 Conclusion

AMLO and TELMI were selected for combination antihypertensive drug therapy. This drug combination is commonly available in the market as conventional tablets for bedtime or morning dosing; but fails to achieve peak concentrations during early morning crisis hours. AMLO exhibits a t_{max} of 6-7 h and TELMI 0.5-1.0 h. Therefore in order to attain desired peak plasma concentration, both the drugs should release with a lag time of 6-7 h. In the present study, we have successfully designed and developed pulsatile capsule of two antihypertensive drugs to mimic the circadian dependent release pattern of change in BP and counteract its early morning rise, without causing a precipitous decline at night. The *in vitro* and *ex vivo* studies indicated that the designed pulsatile capsules were suitable for use as a

chronotherapeutic delivery system for timed release of antihypertensive drugs and could be tailored to synchronize drug release as per needs. This study opens new venture for similar systems to be used as a platform technology for various other disease conditions which follow circadian patterns.

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Figures Legends:

Fig. 1: Assembly of the capsule

Fig. 2: Release profile of AMLO-xylitol granules

Fig. 3: Release profile of TELMI core tablets

Fig. 4: Water uptake studies for erodible tablets of GG and XG

Fig. 5: Comparison of swelling of GG and XG erodible tablets

Fig. 6: Illusion of drug release mechanism of pulsatile capsule

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Tables Legends:

Table 1: Formulation chart of TELMI core tablets

Table 2:Formulation chart for erodible tablets

Table 3: Post-compression parameters of TELMI core tablets

Graphical Abstract