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DESIGN AND EVALUATION OF CHRONOTHERAPEUTIC ANTI-HYPERTENSIVE COMBINATION THERAPY USING MINI-TABLETS TECHNOLOGY

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ABSTRACT: Hypertension or elevated blood pressure (BP) is a serious medical condition that significantly increases the risks of heart, kidney, brain, and other diseases. In persons with normal BP and uncomplicated essential hypertension, BP falls to the lowest levels during the nighttime sleep, and rises suddenly with morning awakening, and reaches the peak during the initial h of diurnal activity. The optimum therapy only results when the right amount of drug is delivered to the right targeted organ at the most suitable time. The present research aimed to develop a chronotherapeutic drug delivery system of combination drugs using mini-tablets technology, which can be administered at bedtime and releases Amlodipine within 2 h followed by the rapid release of Losartan after the lag time of 6 h. The developed mini-tablets were evaluated for compatibility studies, pre-and post-compression parameters. The compatibility studies indicate no chemical reaction, pre-compression parameters indicate good flow properties, and post-compression parameters were within limits. The obtained results of in-vitro studies have shown that Amlodipine and Losartan were not released simultaneously in 0.1N HCL (pH 1.2), as it is observed that only 9.76 ± 1.47 % of Losartan was released at the end of 6 h, which can be considered as lag time. Stability was also found for the developed mini-tablets-filled capsule formulation as per ICH guidelines. Thus, Amlodipine and Losartan can be used as combination products in the form of mini-tablets-filled-capsule formulation for the treatment of Hypertension.

INTRODUCTION: For many of them across the world, medicines have become the order of the day. Hardly there is anyone who has not taken medicine in their life or not any less people who take medicine regularly.

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But many times, the people who take medicines rarely follow the timing of its intake. There are about 60 diseases, including Hypertension, Asthma, Cancer, and Arthritis, for which the drug's effectiveness can be more when taken at the right time of day. Because the optimum therapy only results when the right amount of drug is delivered to the right targeted organ at the most suitable time.

Hence, many adverse effects can be decreased when a drug is not taken when it is actually not required [1]. Through various epidemiological and clinical studies, it has been observed that even the

disease activity levels of a number of clinical disorders such as Arthritis, Asthma, Hypertension, Peptic ulcer *etc.*, have a pattern related to body's biological clock set according to the circadian rhythms. As the normal biological processes are influenced according to the time of the day, thus accordingly, they affect the pathophysiology of the disease and its treatment [1, 3]. Hypertension or elevated blood pressure (BP) is a serious medical condition that significantly increases the risks of heart, kidney, brain, and other diseases. It has been estimated that around 1.13 billion people across the world have hypertension, mostly (two-thirds) living in middle- and low-income countries. Hypertension is a major cause of early death worldwide [4].

In persons with normal BP and uncomplicated essential hypertension, BP falls to the lowest levels during the night time sleep, and rises suddenly with morning awakening, and reaches the peak during the initial hours of diurnal activity. Hence, the administration time of anti-hypertensive drugs is very much important for effective treatment. The chronotherapy concept can better treat hypertension to maintain the drug's highest concentration in the bloodstream during early morning, which minimizes the circadian rhythm of BP and prevents its early morning rise, without causing a sudden decline at night [5, 7]. Chronotherapeutic drug delivery systems may use various drug release mechanisms *e. g.* Osmotic pump mechanisms, Time delay coatings, Pulsincap systems, and matrix systems provide for varying levels throughout the day according to circadian rhythms [8, 9]. Mini-tablets are very small tablets having a diameter equal to or lesser than 3 mm, which can be either placed in sachets or filled into a capsule shell for easy administration.

Mini-tablets have several benefits over single unit larger tablets, such as consistent drug release, uniform clinical performance, more flexibility during the formulation development, and maximum stability on storage [10, 13]. Combination treatment in hypertension as initial therapy may improve drug compliance and simplify treatment, reducing the burden of taking multiple drugs. In addition to increased drug compliance, combination therapy may have other benefits over monotherapy, such as the synergistic mechanism for treating hypertension and reduced adverse effects.

The fixed-dose combination therapy of Amlodipine and Losartan are among the newer combinations of anti-hypertensive drugs that have been extensively studied and hence shown to be effective in the treatment of hypertension [14, 15]. Thus, the present research aimed to develop a chronotherapeutic drug delivery system of combination drugs that can be administered at bedtime and releases Amlodipine within 2 h followed by the rapid release of Losartan after the lag time of 6 h.

MATERIALS AND METHODS:

Materials: Amlodipine and Losartan pure drugs were purchased from Yarrow chem, Mumbai, India. HPMC K4M, HPMC K15M, HPMC K100M, and PVP K 30 polymers were obtained from FMC Biopolymer. pH-sensitive methacrylic acid co-polymer Eudragit® S-100 were supplied as gift samples by Degussa India Pvt. Ltd., Mumbai, India. Microcrystalline cellulose Avicel PH 102 and Aerosil® were purchased from SD Fine Chemicals, Mumbai. Magnesium stearate was purchased from Himedia Chem Lab, Mumbai. Empty HPMC capsules (almost all sizes) were obtained as a gift sample from ACG Associated capsules Pvt. Ltd. Mumbai. All the remaining used materials were of analytical grade.

Pre-formulation Studies:

Fourier Transform Infrared (FTIR) Spectral Analysis: The pure drugs Amlodipine and Losartan, polymers, and drug-polymer physical mixtures used in this experimental composition were evaluated for compatibility by recording the spectra using FTIR Spectrophotometer (Perkin Elmer, spectrum-100, Japan). The spectra were recorded by taking 5% of the sample in potassium bromide (KBr). The sample mixture was blended into a fine powder and finally compressed into KBr pellets at a compaction pressure of 4000 Psi for 2 min. The resolution was 1 $cm^{-1,}$ and the range of scanning was 400 to 4000 cm^{-1}.

Formulation Methods:

Preparation of Matrix Mini-Tablets of Amlodipine (AMMT): The matrix-mini-tablets of Amlodipine were prepared using the direct compression method as per the composition shown in **Table 1**. Initially, pure drug Amlodipine, hydrophilic polymer, and microcrystalline cellulose Avicel PH 102 were passed through 60 mesh sieve,

then weighed as per the composition table and mixed. Then magnesium stearate and aerosil were separately passed through the same sieve and, after weighing were added to the above mixture and thoroughly blended. Then the blended mixture was compressed by using 3 mm round flat-concave punches in a rotary tablet press (Model RSB-4, Rimek mini-press, Karnavathi engineering, Ahmadabad) weighing 25 mg mini-tablets.

TABLE 1: COMPOSITION OF MATRIX-MINI-TABLETS OF AMLODIPINE (AMMT)

Mini-Tablet Batches Code	Amlodipine	HPMC K4M	HPMC K15M	HPMC K100M	PVP K30	Microcrys-Talline Cellulose Avicel PH 102	Magnesium Stearate	Aerosil
AMMT-1	2.5	--	--	--	1	21	0.25	0.25
AMMT-2	2.5	1.25	--	--	1	19.75	0.25	0.25
AMMT-3	2.5	2.5	--	--	1	18.5	0.25	0.25
AMMT-4	2.5	5	--	--	1	16	0.25	0.25
AMMT-5	2.5	7.5	--	--	1	13.5	0.25	0.25
AMMT-6	2.5	10	--	--	1	11	0.25	0.25
AMMT-7	2.5	--	1.25	--	1	19.75	0.25	0.25
AMMT-8	2.5	--	2.5	--	1	18.5	0.25	0.25
AMMT-9	2.5	--	5	--	1	16	0.25	0.25
AMMT-10	2.5	--	7.5	--	1	13.5	0.25	0.25
AMMT-11	2.5	--	10	--	1	11	0.25	0.25
AMMT-12	2.5	--	--	1.25	1	19.75	0.25	0.25
AMMT-13	2.5	--	--	2.5	1	18.5	0.25	0.25
AMMT-14	2.5	--	--	5	1	16	0.25	0.25
AMMT-15	2.5	--	--	7.5	1	13.5	0.25	0.25
AMMT-16	2.5	--	--	10	1	11	0.25	0.25

Note: 1.25= 5%; 2.5=10 %; 5=20%; 7.5=30 %; 10=40% as total weight of each mini-tablet was 25 mg

Preparation of Matrix-Mini-Tablets of Losartan (LMMT): The matrix-mini-tablets of Losartan were prepared using the direct compression method as per the composition shown in **Table 2**. Initially, pure drug Losartan, pH-sensitive polymer, and microcrystalline cellulose Avicel PH 102 were passed through 60 mesh sieve, then weighed as per the composition table and mixed. Then magnesium Stearate and aerosil were separately passed through the same sieve and, after weighing, were added to the above mixture and thoroughly blended. Then the blended mixture was compressed by using 3 mm round flat-concave punches in a rotary tablet press (Model RSB-4, Rimek mini-press, Karnavathi engineering, Ahmadabad) weighing 25 mg mini-Tablets.

TABLE 2: COMPOSITION OF MATRIX-MINI-TABLETS OF LOSARTAN (LMMT)

Mini-Tablet Batches Code	Losartan	Eudragit S-100	Microcrystalline Cellulose Avicel Ph 102	Magnesium Stearate	Aerosil
LMMT-1	3.846	1.25	19.404	0.25	0.25
LMMT-2	3.846	2.5	18.154	0.25	0.25
LMMT-3	3.846	5	15.654	0.25	0.25
LMMT-4	3.846	7.5	13.154	0.25	0.25
LMMT-5	3.846	10	10.654	0.25	0.25
LMMT-6	3.846	12.5	8.154	0.25	0.25
LMMT-7	3.846	15	5.654	0.25	0.25

Note: 1.25= 5%; 2.5=10 %; 5=20%; 7.5=30 %; 10=40%; 12.5=50 %; 15=60% as total weight of each mini-tablet was 25 mg

Preparation of Core-Mini-Tablets of Losartan (LCRMT): The core-mini-tablets of Losartan were prepared using the wet granulation technique. The composition containing Losartan, lactose, intragranular portion of sodium starch glycollate, and PVP K30 as mentioned in Table 3 were passed separately through 60 mesh sieve and blended in dry condition. Then after dry blending the mixture at a slow speed in a double cone blender for a period of 10 min, it was granulated with ethanol. This wet granulated mixture was then passed through a 16 mesh size sieve. The sieved granules were dried in a hot air oven for a period of 1 h at 30 to 35 °C.

These dried granules were allowed to pass through the same 16 mesh size sieve to break the irregularly shaped lumps and finally blended with an extra granular portion of sodium starch glycollate.

The dried granules were then lubricated with magnesium stearate and Aerosil and compressed using 3 mm round flat-concave punches in a rotary tablet press (Karnavati Engineering, Ahmadabad) weighing 25 mg mini-tablets.

TABLE 3: COMPOSITION OF CORE-MINI-TABLETS OF LOSARTAN (LCRMT)

Ingredients	Core mini-tablet (mg)
Losartan	5
Sodium starch glycollate	2.5
PVP K30	1.0
Lactose	16
Magnesiun stearate	0.25
Aerosil	0.25
Total weight (mg)	25.0

Preparation of Coating Solution: Initially, as per the composition shown in **Table 4**, the Eudragit-S100 polymer was added in 50% of the solvents and stirred for a time of 30 - 60 min approximately till the Eudragit-S100 polymer is dissolved completely.

Then, in the remaining solvent mixture triethyl citrate and talc were added and stirred with a high-speed mixer for 10 min. This triethyl citrate and talc solution were poured slowly in stirring motion into the polymer solution and finally allowed to pass through a sieve of 0.5 mm mesh size.

TABLE 4: COMPOSITION OF COATING SOLUTION

Ingredients	Core mini-tablet (mg)
Eudragit-S 100	6.00
Triethyl citrate	0.600
Talc	3.00
Acetone	34.69
Isopropanol	51.42
Water	4.29
Total weight (mg)	100

Preparation of Coated-Mini-Tablets of Losartan (LCMT): Sufficient quantity of Losartan core-mini-tablets along with placebo tablets were coated with 500 ml of coating solution as per the level composition shown below in **Table 5** in a 6" inches lab-scale coating pan (Make- United Technologies Company, Mumbai, India). The operating parameters for coating pan were - tablets charge 100 g; inlet air temperature 45 ± 5 °C; preheating temperature 40 ± 5 °C; preheating time 20 min; atomization pressure 2.0 bar, nozzle diameter 1.0 mm, spray rate 8 – 10 ml/min.

TABLE 5: COMPOSITION OF COATED MINI-TABLETS OF LOSARTAN (LCMT)

Mini-tablet batches Code	Coating level (%)
LCMT-1	5
LCMT-2	10
LCMT-3	15
LCMT-4	20

Preparation of Mini-Tablets-Filled Capsule (MTFC) Formulation: Based on *in-vitro* results of AMMT, LMMT, and LCMT batches, MTFC formulation was prepared by filling optimized matrix-mini-tablets equivalent to 5 mg of Amlodipine (*i.e.* 2 mini-tablets) and optimized coated-mini-tablets equivalent to 50 mg of Losartan (*i.e.* 13 mini-tablets) into a size "0" HPMC capsule as shown in **Fig 1**. The developed MTFC formulation of Amlodipine and Losartan was then stored at room temperature until testing.

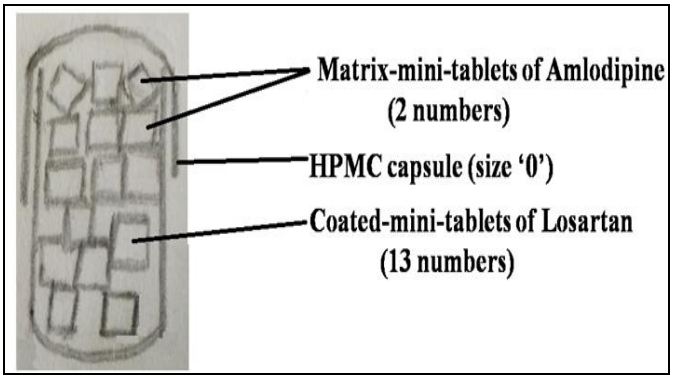

FIG. 1: SCHEMATIC REPRESENTATION OF MINI-TABLETS-FILLED CAPSULE FORMULATION OF AMLODIPINE AND LOSARTAN

Evaluation Methods:

Pre-Compression Parameters: The prepared powder blends ready for compression containing drug, polymers, and various excipients were evaluated for pre-compression parameters to study their flow properties and to maintain uniformity of matrix-mini-tablets weight.

Angle of Repose (Ɵ): The angle of repose for the prepared powder blend was determined by taking accurately weighed powder blend into the funnel. The height of the funnel was adjusted as such that the tip of the funnel should touch the apex of the blend. Then the blend was allowed to flow through the funnel freely on to the surface.

Then from the formed powder cone, height and radius was measured, and the angle of repose was calculated using the following equation [16].

$$\tan \theta = h/r$$

Where, h and r are the height and radius of the powder cone, respectively.

Bulk density and tapped density: For the powder blend, both loose bulk density (LBD) and tapped bulk density (TBD) were determined. Weighed quantity of 2 gm of powder blend from each batch, previously shaking to break any formation of agglomerates, was introduced into 10 ml of measuring cylinder. By noting the initial volume, the cylinder was allowed to fall under its own weight on to a hard surface from the height of 2.5 cm at 2-sec intervals. Tapping was continued until no further change in volume was noted. LBD and TBD were calculated using the following equations [16].

LBD = Weight of the powder blend/ Untapped Volume of the packing

Hausner = Pt/Pd

TBD = Weight of the powder blend/ Tapped Volume of the packing

Carrs's Index (%) = TBD – LBD/TBD

Hausner's Ratio: Hausner's ratio is considered as an indirect index of ease of powder flow. It is calculated by using the following equation [16].

Hausner ratio = Where, $W_{initial}$ t is tapped density and $W_{initial}$ d is bulk density. Carr's

Compressibility Index: The compressibility index of the powder blend was determined by Carr's compressibility index. Carr's Index (%) can be calculated by using the following equation [16].

Carr's Index (%) = LBD – TBD/TBD × 100

Post-Compression Parameters: The prepared matrix-mini-tablets were evaluated for post-compression parameters to study their physic-chemical properties.

Hardness Test: The hardness of the matrix-mini-tablets was determined using a Pfizer hardness tester. From each formulation batch, three matrix-mini-tablets were randomly taken, and the values were calculated [16].

Friability Test: The test was performed by initially weighing ($W_{initial}$) twenty matrix-mini-tablets and then transferring them into a Veego friabilator. The friabilator was operated at 25 rpm and run up to 100 revolutions. Then the mini-tablets were weighed again (W_{final}) [16].

The % friability was then calculated by using the following equation.

Weight Variation Test: From each formulation batch, twenty mini-tablets were randomly taken and weighed individually to check the weight variation [16].

Uniformity of Thickness: From each formulation batch, six matrix-mini-tablets were randomly taken and was measured for thickness using screw gauge [16].

Drug Content Uniformity:

For Matrix-Mini-Tablets of Amlodipine: Ten matrix-mini-tablets were crushed in a mortar and then weighed powder equivalent to 5 mg of Amlodipine was extracted in pH 1.2 HCL buffer solution. The extracted solution was filtered through a Millipore filter of 0.45 μm pore size, and the content of the drug was determined spectro-photometrically at a λ_{max} of 295 nm after suitable dilution. For matrix-mini-tablets and coated-mini-tablets of Losartan: Twenty matrix-mini-tablets/ coated-mini-tablets were crushed in a mortar and then weighed powder equivalent to 50 mg of Losartan was extracted in pH 7.4 phosphate buffer solution. The extracted solution was filtered through a Millipore filter of 0.45μm pore size, and the content of the drug was determined spectro-photometrically at a λ_{max} of 236 nm after suitable dilution.

***In-vitro* Dissolution Studies:** Separate *in-vitro* dissolution testing was carried out in order to select the optimized AMMT, LMMT, and LCMT batches for developing the mini-tablets-filled capsule formulation as per the desired criteria. An evaluation was done by using the USP XXIII dissolution test apparatus (paddle method). Dissolution temperature and the speed of rotation were maintained at 37 ± 0.5 °C and 100 rpm,

respectively, for all the mini-tablet batches and mini-tablets-filled capsule formulation. For AMMT batches, dissolution testing was carried out in 750 ml of 1.2 pH media for a period of 2 h.

Whereas for LMMT and LCMT batches, in order to match the increased pH changes along the Gastrointestinal tract, four dissolution medias with pH 1.2, 6.5, 6.8, and 7.2 were used sequentially. These four media represents the stomach, proximal and distal parts of the small intestine, and terminal ileum, respectively. During dissolution testing, 750 ml of 1.2 pH media was used for the first two hours and was then continued with 900 ml of 6.5, 6.8, and 7.4 pH phosphate buffers for a period of 1, 2, and subsequent hours, respectively.

Moreover, dissolution testing evaluation was also carried out for the complete mini-tablets-filled capsule formulation filled with both AMMT and LCMT to assess the in-vitro release of both the drugs (Amlodipine and Losartan) when administered in combination. As Amlodipine matrix-mini-tablets were targeted in stomach and Losartan as intestinal targeted mini-tablets. For this purpose, the dissolution study was initially carried out in 750 ml of 1.2 pH media for first two hours. After 2 h, Losartan mini-tablets were removed from the dissolution apparatus and transferred into another USP XXIII dissolution test apparatus containing 900 ml of pH 6.5, 6.8 and 7.2 phosphate buffers for a period of 1, 2 and 1 h respectively. For all the AMMT batches, 5 ml of dissolution media was withdrawn at fixed time intervals (*i.e*. 0, 30, 60, 90 and 120 min) and was then replaced with fresh respective dissolution media.

Whereas, for all the LMMT batches and mini-tablets-filled capsule formulation, 5 ml of dissolution media was withdrawn at fixed time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12) and was then replaced with fresh respective dissolution media. The withdrawn samples of Amlodipine were analyzed at a wavelength region of 295 nm, and withdrawn samples of Losartan were analyzed at 236 nm by UV-absorption spectroscopy, and the drug release percentage was calculated over the sampling time intervals. For all AMMT, LMMT, and LCMT batches and mini-tablets-filled-capsule formulation, the *in-vitro* release profile was determined thrice, and the average values were presented in the form of graphical representation [17, 20].

Stability Studies: The stability studies for optimized mini-tablets-filled capsule formulation were performed at both room temperature and accelerated stability conditions. The room temperature (RT) storage conditions were kept at 30 ± 2 °C and 65 ± 5% relative humidity (RH), and accelerated stability conditions were stored at 40 ±2 °C and 75 ± 5% RH in a stability chamber. At regular intervals of time of 3 and 6 months, samples were withdrawn from the stability chamber and were checked for physical parameters such as Appearance, Weight variation, Hardness, Thickness, Friability, Drug content, and *in-vitro* drug release profile [21, 22].

RESULTS AND DISCUSSION:

Pre-formulation Studies:

FT-IR Studies: In order to check drug-polymers compatibility, the spectra of pure drugs (Amlodipine, Losartan), polymers, and their respective physical mixtures in AMMT and LMMT batches were recorded as shown in **Fig 2**. In the AMMT batches, Amlodipine was used as the model drug and HPMC-K4M, HPMC-K15M, HPMC-K100M, and PVP-K30, as polymers, respectively. Amlodipine has shown amine, ethyl ester, salts of sulphonic acid, and aliphatic esters stretchings due to the presence of characteristic peaks at 3095.64 cm^{-1}, 1447.45 cm^{-1}, 1286.54, and 1104.49 $cm^{-1,}$ respectively.

These are all the characteristic peaks of Amlodipine. The HPMC K4M polymer has shown -OH, -C-H, and -C=O stretchings due to the presence of characteristic peaks at 3474.52 cm^{-1}, 2832.53 $cm^{-1,}$ and 1378.63 $cm^{-1,}$ respectively. The HPMC K15M polymer has shown -OH, -C-H, and -C=O stretchings due to the presence of characteristic peaks at 3471.47 cm^{-1}, 2839.59 $cm^{-1,}$ and 1371.55 $cm^{-1,}$ respectively. The HPMC-K100M polymer has shown -OH, -C-H, and -C=O stretchings due to the presence of characteristic peaks at 3479.64 cm^{-1}, 2816.57 $cm^{-1,}$ and 1386.84 $cm^{-1,}$ respectively. Whereas the PVP-K30 polymer spectra have shown -CH2, -C-H, -C=O, and -C-N stretchings due to the presence of characteristic peaks at 2947.05 cm^{-1}, 2158.18 cm^{-1}, 1705.22 $cm^{-1,}$ and 1415.48 $cm^{-1,}$ respectively.

Moreover, in the LMMT mini-tablet batches, as shown in **Fig. 3**, Losartan was used as the model drug and Eudragit-S100 as polymer. Losartan potassium has shown -CH, -NH, and -C-N stretchings due to the presence of characteristic peaks at 3012.14 cm^{-1}, 3296.28 $cm^{-1,}$ and 1312.64 $cm^{-1,}$ respectively. Eudragit-S100 polymer spectra also shows similar -OH, $-OCH_3$, $-CH_3$, and -C=O stretchings due to the presence of characteristic Peaks at 3225 cm^{-1}, 2998 cm^{-1} 2, 953 $cm^{-1,}$ and 1727 $cm^{-1,}$ respectively. Finally, when the physical mixture spectra of pure drugs (Amlodipine and Losartan) were recorded with their respective polymers as per the formulation tables, it was found that their respective higher spectra have also shown all the peaks of pure drugs and polymers. None of the peaks was absent, as they were found to be intact. Thus, it confirms that the combination of the used drugs and polymers in the mini-tablet batches (both AMMT and LMMT) can be suitable for designing a mini-tablets-filled-capsule formulation needed for its desired therapeutic purpose.

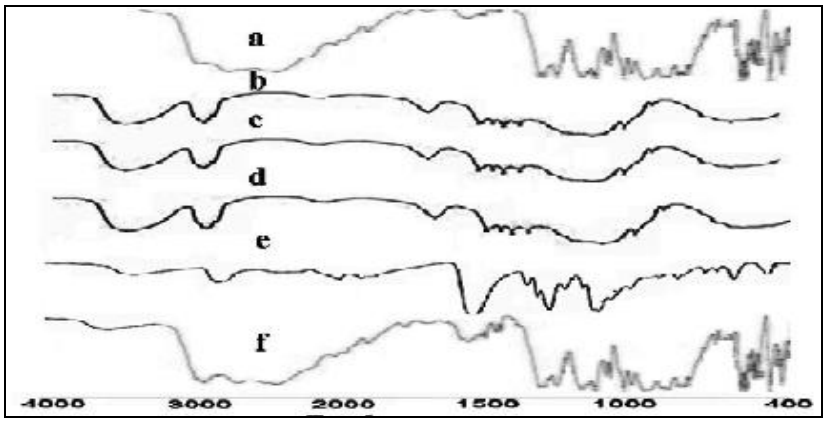

FIG. 2: FTIR SPECTRA OF A) AMLODIPINE B) HPMC K4M B) HPMC K15M C) HPMCK 100M D) PVP K-30E) PHYSICAL MIXTURE OF AMLODIPINE+HPMC K4M+HPMC K15M +HPMC K100M+PVP K-30

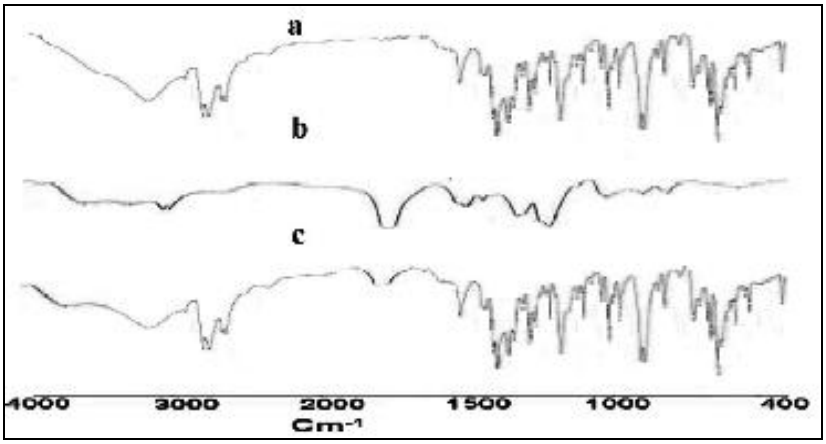

FIG. 3: FTIR SPECTRA OF A) LOSARTAN B) EUDRAGIT S-100 E) PHYSICAL MIXTURE OF LOSARTAN+ EUDRAGIT S-100

Evaluation of the Prepared Powder Blends of Amlodipine and Losartan: The Angle of repose values for the powdered blend of AMMT, LMMT, and LCRMT mini-tablets was found to range between 22°.09' ± 0 .21 to 24°.98'± 0.18. The LBD and TBD values were found to range between 0.522 ± 0.01 to 0.542 ± 0.01 and 0.585 ± 0.01 to 0.608 ± 0.01gm/cc respectively. The Carr's compressibility index values were found to range between 13.09 ± 0.47 to 14.96 ± 0.40 %. The Hausner's ratio values were found to range between 1.15 ± 0.01 to 1.17 ± 0.01. As, angle of repose values were found to be less than 30º, Carr's compressibility index values were found to be less than 15% and Hausner's ratio values were also found to be lesser than 1.25, hence it indicates better flow properties. Thus, the prepared powder blend was found to exhibit good flow properties as evident from the results shown in **Table 6**.

TABLE 6: RESULTS OF PHYSICAL EVALUATION OF PRE-COMPRESSION BLEND OF MINI-TABLETS

Mini-Tablet Batches Code	Angle of Repose (°) Mean ± SD, N=3	Bulk Density (G/Cc) Mean ± SD, N=3	Tapped Density (G/Cc) Mean± SD, N=3	Carr's Index (%) Mean ± SD, N=3	Hausner's Ratio Mean ± SD, N=3
AMMT-1	24°.47'±0.10	0.525 ± 0.01	0.608 ± 0.01	13.65 ±0.63	1.15±0.01
AMMT-2	24°.26'±0.14	0.531 ± 0.01	0.598 ± 0.01	13.09 ±0.47	1.15±0.01
AMMT-3	23°.60'±0.13	0.522 ± 0.01	0.588 ± 0.01	14.84 ±0.82	1.17±0.01
AMMT-4	24°.88'±0.11	0.534 ± 0.01	0.602 ± 0.01	13.59 ±0.38	1.15±0.01
AMMT-5	24°.98'±0.18	0.527 ± 0.01	0.585 ± 0.01	14.44 ±0.57	1.16±0.01
AMMT-6	24°.12'±0.12	0.538 ± 0.01	0.594 ± 0.01	13.50 ±0.89	1.15±0.01
AMMT-7	22°.91'±0.15	0.539 ± 0.01	0.604 ± 0.01	14.17 ±0.74	1.16±0.01
AMMT-8	24°.44'±0.12	0.534 ± 0.01	0.601 ± 0.01	14.96 ±0.40	1.17±0.01
AMMT-9	22°.33'±0.18	0.529 ± 0.01	0.595 ± 0.01	14.53 ±0.93	1.17±0.01
AMMT-10	23°.79'±0.24	0.528 ± 0.01	0.599 ± 0.01	14.42 ±0.58	1.16±0.01
AMMT-11	24°.85'±0.13	0.540 ± 0.01	0.608 ± 0.01	13.73 ±0.74	1.15±0.01
AMMT-12	23°.93'±0.19	0.532 ± 0.01	0.597 ± 0.01	14.60 ±0.49	1.17±0.01
AMMT-13	23°.20'±0.15	0.538 ± 0.01	0.605 ± 0.01	14.73 ±0.32	1.17±0.00
AMMT-14	22°.57'±0.12	0.542 ± 0.01	0.607 ± 0.01	14.51 ±0.57	1.16±0.01
AMMT-15	24°.41'±0.18	0.535 ± 0.01	0.593 ± 0.01	14.53 ±0.86	1.17±0.01
AMMT-16	23°.39'±0.16	0.528 ± 0.01	0.590 ± 0.01	14.42 ±0.95	1.16±0.01
LMMT-1	22°.17'±0.12	0.530 ± 0.01	0.597 ± 0.01	14.79 ±0.54	1.17±0.01
LMMT-2	24°.38'±0.21	0.522 ± 0.01	0.586 ± 0.01	14.28±0.32	1.16±0.00
LMMT-3	22°.62'±0.10	0.529 ± 0.01	0.586 ± 0.01	14.26±0.56	1.16±0.01
LMMT-4	24°.40'±0.14	0.537 ± 0.01	0.605 ± 0.01	14.49±0.70	1.16±0.01
LMMT-5	24°.32'±0.26	0.526 ± 0.01	0.587 ± 0.01	14.61±0.92	1.17±0.01
LMMT-6	22°.09'±0.21	0.541± 0.01	0.610 ± 0.01	14.53±0.88	1.17±0.01
LMMT-7	23°.37'±0.16	0.527 ± 0.01	0.603 ± 0.01	14.72±0.49	1.17±0.00
LCRMT	24°.89'±0.12	0.524 ± 0.01	0.599 ± 0.01	14.09±0.64	1.16±0.01

Evaluation of Prepared Mini-Tablets of Amlodipine and Losartan: The weight variation values for all the prepared mini-tablets were found to range between 23 ± 0.12 to 26 ± 0.18 mg. The pharmacopoeial limit for percentage deviation of tablets of 130 mg or less is ± 10%, and all the AMMT, LMMMT, and LCRMT batches were found to pass as per the given specifications in Indian pharmacopoeia. The Hardness values were found to be uniform and were range between 2.22 ± 0.08 to 2.38 ± 0.12 kg.

The values of Friability were found to range between 0.39 ± 0.08 to 0.66 ± 0.12%, and they have also shown that mini-tablets have got sufficient strength. The Thickness values were found to range between 2.12 ± 0.01 to 2.34 ± 0.01 mm. Excellent drug content uniformity was found in all the mini-tablet batches, as their values were found to range between 97.05 ± 0.09 to 99.92 ± 0.16 %, which is more than 95%. Thus, all the physicochemical properties of mini-tablet batches were found to be satisfactory as shown in **Table 7**.

TABLE 7: RESULTS OF POST-COMPRESSION EVALUATION OF THE PREPARED MINI-TABLETS

Mini-Tablet Batches Code	Weight Variation (Mg) (Mean ± SD), N=20	Hardness (Kg) (Mean ± SD), N=6	Thickness (Mm) (Mean ± SD), N=6	Friability (%) (Mean ± SD), N=20	% Drug Content (Mean ± SD), N=3
AMMT-1	24 ± 0.14	2.26 ± 0.07	2.19±0.01	0.52±0.09	99.47±0.08
AMMT-2	23 ± 0.12	2.32 ± 0.10	2.27±0.01	0.46±0.06	98.68±0.12
AMMT-3	24 ± 0.15	2.29 ± 0.06	2.14±0.01	0.51±0.11	98.92±0.17
AMMT-4	24 ± 0.17	2.22 ± 0.08	2.19±0.01	0.39±0.08	99.83±0.10
AMMT-5	25 ± 0.13	2.30 ± 0.14	2.24±0.01	0.55±0.06	97.05±0.09
AMMT-6	23 ± 0.16	2.32 ± 0.09	2.18±0.01	0.54±0.09	99.30±0.16
AMMT-7	24 ± 0.12	2.36 ± 0.15	2.23±0.01	0.44±0.01	98.71±0.12
AMMT-8	26 ± 0.15	2.31 ± 0.14	2.29±0.01	0.50±0.06	99.66±0.07
AMMT-9	24 ± 0.16	2.27 ± 0.10	2.25±0.01	0.48±0.09	99.28±0.13
AMMT-10	26 ± 0.10	2.33 ± 0.11	2.34±0.01	0.66±0.12	98.39±0.09
AMMT-11	24 ± 0.15	2.25 ± 0.16	2.26±0.01	0.49±0.09	97.19±0.16
AMMT-12	23 ± 0.14	2.26 ± 0.08	2.18±0.01	0.61±0.11	98.59±0.06

AMMT-13	24 ± 0.16	2.29 ± 0.12	2.25±0.01	0.44±0.08	99.85±0.10
AMMT-14	24 ± 0.18	2.35 ± 0.11	2.20±0.01	0.59±0.05	99.04±0.14
AMMT-15	25 ± 0.10	2.37 ± 0.16	2.19±0.01	0.46±0.09	99.49±0.12
AMMT-16	26 ± 0.18	2.34 ± 0.13	2.32±0.01	0.55±0.08	97.98±0.09
LMMT-1	24 ± 0.12	2.29 ± 0.07	2.27±0.01	0.50±0.05	98.44±0.18
LMMT-2	25 ± 0.10	2.36 ± 0.10	2.25±0.01	0.48±0.07	99.84±0.15
LMMT-3	26 ± 0.17	2.30 ± 0.15	2.30±0.01	0.59±0.12	98.78±0.10
LMMT-4	25 ± 0.14	2.38 ± 0.12	2.25±0.01	0.53±0.09	99.92±0.16
LMMT-5	26 ± 0.18	2.32± 0.09	2.29±0.01	0.62±0.06	98.49±0.14
LMMT-6	25 ± 0.15	2.29 ± 0.17	2.31±0.01	0.49±0.07	99.70±0.12
LMMT-7	26 ± 0.14	2.34 ± 0.10	2.33±0.01	0.56±0.09	97.71±0.09
LCRMT	24 ± 0.18	2.17 ± 0.11	2.12±0.01	0.63±0.10	99.46±0.17

***In-vitro* Dissolution Studies for Amlodipine Matrix Mini-Tablet Batches (AMMT):** The AMMT batches were prepared by varying concentration ratios (5%, 10%, 20%, 30%) and viscosity grades of HPMC polymers (HPMC K4M, HPMC K15M, HPMC K100M) as release retardant agent, so as to sustain the release of Amlodipine in the 0.1 HCl (pH 1.2) for at least 2 h. PVP K30 was incorporated as a binder in matrix mini-tablets. For all the AMMT batches, mini-tablets equivalent to 5 mg of Amlodipine (*i.e*. 2 number) were filled into the size "4" HPMC capsule. Further, all the AMMT filled capsule batches were separately subjected to dissolution testing in order to find out the effect of varying concentration ratios and viscosity grades of HPMC polymers in mini-tablets. The test objective was to identify the most suitable optimized AMMT batch which can be combined with the optimized LMMT or LCMT batch for preparing the novel mini-tablets-filled-capsule formulation.

From the in-vitro dissolution results as represented in **Fig. 4A**, it was found that only the mini-tablet batches AMMT-6 (with 40% HPMC K4M), AMMT-10 (with 30% HPMC K15M), AMMT-11 (with 40% HPMC K15M), AMMT-15 (with 30% HPMC K100M), AMMT-16 (with 40% HPMC K100M) sustained the release of Amlodipine at the end of 2 h. The dissolution testing results have also shown that as the viscosity and concentration of the hydrophilic polymer in mini-tablets were increasing, the release rate of Amlodipine was decreased. This is because the increased polymer viscosity has retarded the drug release. Hence, after observing the release profile of all the AMMT batches, AMMT-16 was considered as the most suitable batch because it released a maximum portion of the drug (*i.e*. 99.73 ± 0.87%) at the end of 2 h when compared to all the remaining batches. Hence, HPMC-K100M polymer when incorporated in mini-tablets in 40% concentration, has sustained the release of Amlodipine in pH 1.2 HCl for a period of 2 h and thus was selected for developing a mini-tablets-filled capsule formulation.

***In-vitro* Dissolution Testing for Losartan Matrix Mini-Tablet Batches (LMMT):** The LMMT batches were prepared by varying concentrations of Eudragit S100 (5%, 10%, 20%, 30%, 40%, 50%, 60%) as pH-dependent polymer, so as to prevent the release of Losartan in the pH of the stomach and to release only in small intestinal pH. For all the LMMT batches, mini-tablets equivalent to 50 mg of Losartan (*i.e*. 13 mini-tablets) were filled into size "0" HPMC capsule. Further, all the LMMT filled capsule batches were individually subjected to *in-vitro* dissolution testing in order to find out the effect of various concentrations of pH-dependent polymer in mini-tablet batches.

The objective was to select the most optimized LMMT batch, which can be combined with the optimized AMMT batch so as to prepare the novel mini-tablets-filled-capsule formulation. From the results of *in-vitro* dissolution studies as represented in **Fig. 4B**, it was found that all the LMMT batches released Losartan after a lag time of 124 to 322 min and 97.18 ± 0.75 to 99.97 ± 1.03% of Losartan at the end of 12 h, respectively. The results have also shown that as the concentration of Eudragit-S100 polymer in mini-tablets was increasing, the release rate of Losartan was decreasing. It was also observed that all the 7 LMMT mini-tablet batches prepared with 5 to 60% concentration of Eudragit-S100 polymer were not able to release Losartan after a lag time of 6 h. The maximum lag time of 322 min (*i.e*. 5 h 22 min) was obtained by LMMT-7 batch containing 60% concentration of Eudragit-S100 polymer. This is because the Eudragit-S100 polymer has low solubility in pH 1.2, 6.5, 6.8

buffers and high solubility in pH 7.0 or above buffers. Finally, after observing the release profile of all the LMMT batches, it was further planned to develop to coated mini-tablets of Losartan in order to achieve a lag time of 6 h.

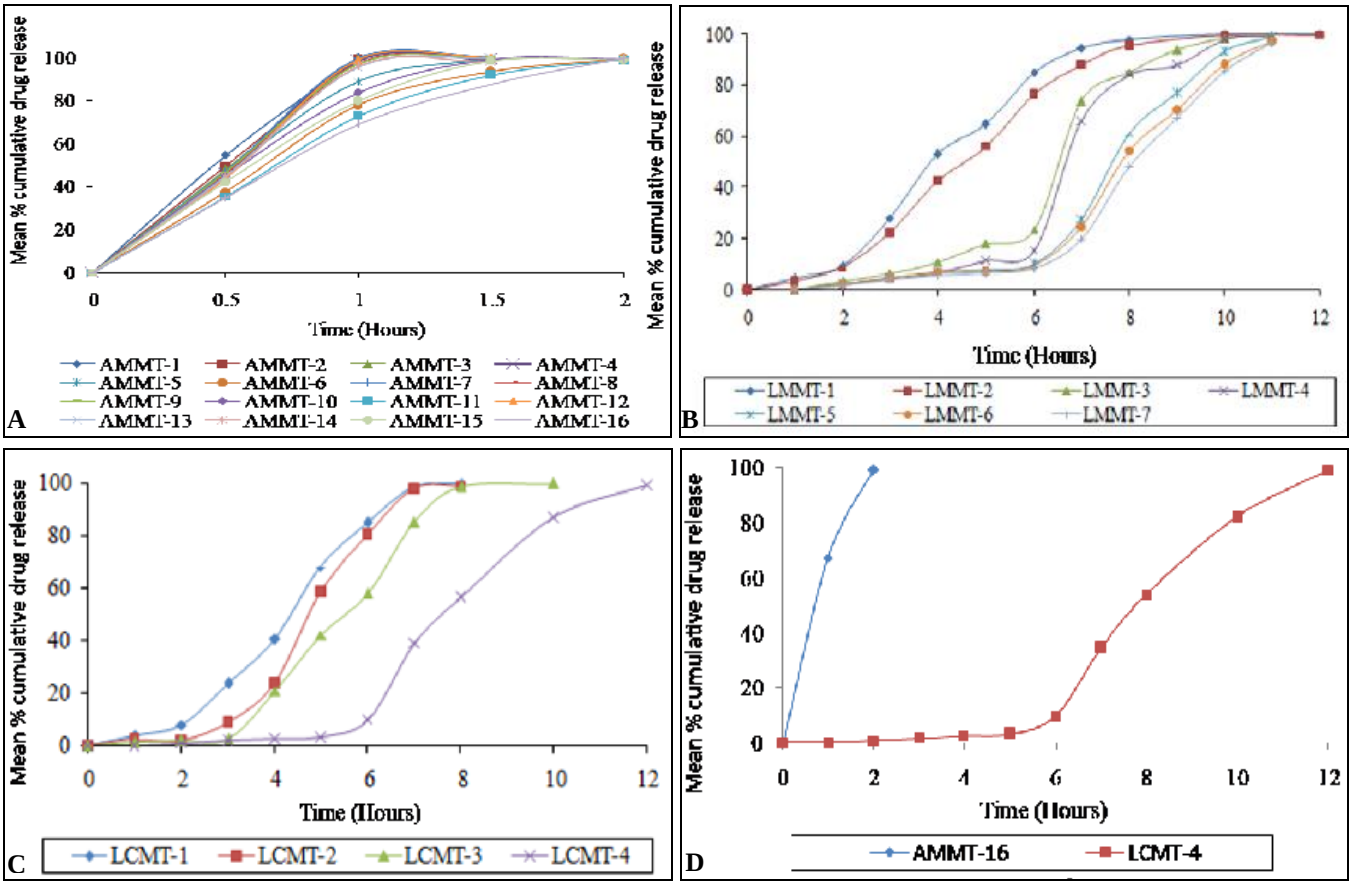

FIG. 3: *IN-VITRO* DISSOLUTION PROFILE OF A) AMLODIPINE MATRIX-MINI-TABLETS B) LOSARTAN MATRIX-MINI-TABLETS C) LOSARTAN COATED-MINI-TABLETS D) MINI-TABLETS-FILLED-CAPSULE FORMULATION OF AMLODIPINE AND LOSARTAN

***In-vitro* Dissolution Testing for Losartan Coated Mini-Tablet Batches (LCMT):** The LCMT batches were prepared by coating the core tablets with varying concentrations of Eudragit-S100 polymer (5%, 10%, 15%, 20%) as pH-dependent polymer so as to prevent the release of Losartan in the pH of the stomach, small intestine and to release only in the colon after a lag time of 6 h. For all the LCMT batches, mini-tablets equivalent to 50 mg of Losartan (*i.e.* 13 mini-tablets) were filled into size "0" HPMC capsule. Further, coated mini-tablets of Losartan were individually subjected to *in-vitro* dissolution testing in order to find out the effect of various coating concentrations of pH-dependent polymer on mini-tablets.

The objective was to select the most optimized LCMT batch, which can be combined with the optimized AMMT batch so as to prepare the novel mini-tablets-filled-capsule formulation. From the results of *in-vitro* dissolution studies as represented in **Fig. 4C**, it was found that the LCMT batches released Losartan after a lag time of 132 to 364 min and 99.93 ± 0.96 to 99.22 ± 0.83 % of Losartan at the end of 12 h. The results have also shown that as the coating concentration of Eudragit-S100 polymer in mini-tablets was increasing, the release rate of Losartan was decreasing.

It was also observed that the first 3 LCMT batches coated with 5 to 15% concentration of Eudragit-S100 polymer were not able to release Losartan after a lag time of 6 h. The maximum lag time of 364 min (*i.e.*, 6 h 4 min) was obtained by LCMT-4 batch containing 20% concentration of Eudragit-S100 polymer. This is because the Eudragit-S100 polymer has low solubility in pH 1.2, 6.5, 6.8 buffers and high solubility in pH 7.0 or above buffers. But still, only high concentration (*i.e.*, 20%) of Eudragit S-100 polymer was able to

prevent the release of Losartan in the pH of stomach, proximal and distal parts of the small intestine, and further 1 hour in terminal ileum also. Finally, after observing the release profile of all the LCMT batches, LCMT-4 was considered as the optimized batch and thus was selected for developing a mini-tablets-filled capsule formulation.

***In-vitro* Dissolution Testing of Mini-Tablets-Filled-Capsule Formulation of Amlodipine and Losartan (MTFC):** Finally, after observing the separate *in-vitro* dissolution testing results of Amlodipine and Losartan mini-tablet batches, AMMT-16 and LCMT-4 were considered as optimized, and 2 numbers of AMMT-16 and 13 numbers of LCMT-4 were filled into a size "0" HPMC capsule for developing a mini-tablets-filled-capsule formulation. Further, the entire capsule formulation was also subjected to further in-vitro dissolution testing.

From the results, as represented in **Fig. 4D**, it was found that capsule formulation released 99.05 ± 0.87% of Amlodipine at the end of 2 h and 9.76 ± 1.47%, 98.94 ± 0.22% of Losartan was released at the end of 6 and 12 h respectively. The developed capsule formulation has successfully released Amlodipine and Losartan in the pH of the stomach and colon, respectively as observed from the *in-vitro* results. Moreover, Amlodipine and Losartan were not released simultaneously in 0.1 NHCL (pH 1.2), as it is observed that only 9.76 ± 1.47% of Losartan was released at the end of 6 h, which can be considered as lag time. Thus, Amlodipine and Losartan can be used as combination products in the form of mini-tablets-filled-capsule formulation for the chronotherapeutic treatment of Hypertension.

Stability Studies: The obtained results of stability studies at both room temperature and accelerated stability revealed that there was very small variation (*i.e.*, < 1%) in post-compression parameters, drug content, and in-vitro drug release profile of the optimized mini-tablets-filled-capsule formulation. The observed results have not shown any significant changes in all the parameters of Amlodipine matrix mini-tablets (AMMT-16) and Losartan coated-mini-tablets (LCMT-4) batches during the 6 month period of study. Thus for the developed mini-tablets-filled-capsule formulation, stability was found as per ICH guidelines.

CONCLUSION: The present research aimed to develop a chronotherapeutic drug delivery system of combination drugs using mini-tablets technology which can be administered at bedtime and releases Amlodipine within 2 h followed by the rapid release of Losartan after the lag time of 6 h. The obtained results of *in-vitro* studies have shown that Amlodipine and Losartan were not released simultaneously in 0.1N HCL (pH 1.2), as it is observed that only 9.76 ± 1.47% of Losartan was released at the end of 6 h, which can be considered as lag time. Thus, Amlodipine and Losartan can be used as combination products in the form of mini-tablets-filled-capsule formulation for the chrono-therapeutic treatment of Hypertension.

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