Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 10 June 2026
Revised 20 July 2026
Accepted 05 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (13s): 546–559

KEYWORDS:
Bioavailability Enhancement, Type 2 Diabetes Mellitus (T2DM), Chronotherapy, Pulsatile Drug Delivery System (PDDS), Linagliptin, Metformin Hydrochloride.

Temporal Precision in Antidiabetic Drug Delivery: Development and Evaluation of a Dual-Drug Pulsatile System for Metformin and Linagliptin

Ravali V[1], Balaji P*[2]

[1]Research scholar, School of Pharmaceutical Sciences, VISTAS, Pallavaram, Chennai, Tamil Nadu, India, 600117
*[2]Professor, Department of Pharmacology, School of pharmaceutical sciences, VISTAS, Pallavaram, Chennai, Tamil Nadu, India, 600117

ABSTRACT: This study showed that ML-PULSE which is a new pulsatile drug delivery system (PDDS) applied the concept of bioavailability to release drug in time with circadian rhythms to regulate Type 2 diabetes. Metformin HCl (MP4) and Linagliptin (LP5) were taken as model drugs. Preformulation analysis confirmed high solubility of Metformin and moderate solubility of Linagliptin which were then applied to carry out formulation modifications. The UV-Vis absorbance of the two drugs at 232 nm was similar and thus was accurate in quantification. Optimal formulations were derived, and the particle sizes were nanometric, entrapment efficiency was very high, and lag times were precise (3 hours MP4, 2.5 hours LP5), and about 88% of the drug was released in 12 hours. The in-vitro research design depicted zero-order release of Metformin and Korsmeyer-Peppas kinetics of Linagliptin. Pharmacokinetic studies in diabetic rats showed that AUC 0-infinity had gone up significantly, Tmax was delayed, half-life was extended and the rate of elimination was decreased which was in support of chronotherapy. There was in-vivo efficacy which showed considerable improvement in glucose regulation, insulin sensitivity and tissue protection. The results of the stability test were confirmed and determined to be very intact even in accelerated and long-term environment. ML-PULSE was found to be a potential two-drug PDDS which offered more glycemic control.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels due to a failure in insulin secretion, insulin action or both. It may cause problems in different organ systems including cardiovascular disease, neuropathy, retinopathy and nephropathy. There are two major types: Type 1 diabetes mellitus (T1DM) is an autoimmune disease that requires insulin treatment for life and Type 2 diabetes mellitus (T2DM) is a progressive disease, associated with insulin resistance and beta-cell dysfunction, and often caused by obesity, physical inactivity, and unhealthy eating habits [1]. T2DM is responsible for more than 90% of diabetes cases worldwide and is a significant public health problem. According to the International Diabetes Federation, there were 537 million adults affected in 2021 and this is projected to increase to 783 million by 2045 [2]. However, many people do not reach optimal glycemic control despite efforts to treat it because of irregular drug levels, side effects such as hypoglycemia, and low compliance rates due to the complexity of the treatment plan [3].

The body's circadian rhythm is not met by current systems of drug delivery, especially the early morning glucose rise (dawn phenomenon) that is linked to poor glycemic control in T2DM. Pulsatile drug delivery systems (PDDS) are systems that

release drugs at predetermined times; they are a novel approach for mimicking the insulin secretion in the body and improve the bioavailability of the drug while reducing the side effects [4]. PDDS can help to decrease postprandial glucose spikes and increase patient compliance by lowering the number of doses in diabetes management. In recent years, new nano-technology, polymer technology and biomaterials have led to the creation of advanced PDDS: multi-layered tablets, hydrogel matrices and nanoencapsulated systems which enable controlled and responsive drug release [5–7]. But, there are still challenges such as formulation optimization, selection of responsive polymers, understanding the dual releasing behavior of these formulations, and scalability of manufacturing [8]. The resolution of these issues is not only critical to bringing PDDS from the bench to the bedside but it is also important for the success of the project. The study here was directed towards the formulation and evaluation development of ML-PULSE, a dual-drug PDDS for Metformin HCl and Linagliptin for the purpose of providing a chronopharmacological synchronization, improved bioavailability and continuous therapeutic effect for the treatment of glycemic control in T2DM [9].

2. MATERIALS AND METHODS

2.1 Materials and Instruments Used

Excipients including PVP K30, HPMC K4M, Ethyl Cellulose, Aerosil, MCC and Crospovidone were used in conjunction with the active ingredients, metformin HCl (USP grade) and Linagliptin (analytical grade). SOLUBILITY STUDY IN IPA, Ethanol, Methanol, Acetone and DMSO. They were used, among others: UV-Visible Spectrophotometer (Shimadzu UV-1800); HPLC (Agilent 1200 Series) with a C18 column; DSC; FTIR; fluidized bed processors for coating the beads; a DLS Analyzer for measuring the particle size. Wistar rats (180-220g) were used for in vivo studies.

2.2 Drug-Excipient Compatibility Studies

The thermal behavior, as well as possible interactions of Metformin HCl, Linagliptin, and excipient (PVP, Aerosil, HPMC K4M, Ethyl Cellulose) in the presence of nitrogen were assessed using DSC, allowing them to be compatible in formulation. Stability of the chemicals was also supported by FTIR spectroscopy (4000-400 cm^{-1}) because no great difference in the typical drug peaks was noticed, which implied the excipients did not chemically react with the drugs [20-24].

2.3 Preparation of ML-PULSE Pulsatile Drug Delivery System

The ML-PULSE system was prepared using a 2^3 factorial design, focusing on HPMC K4M (A), Ethyl Cellulose EC7 CPS (B), and DOP (C) at low and high levels across eight formulations. Immediate-release Metformin granules were prepared by blending Metformin HCl with soluble starch and crospovidone, wet-granulating the mixture with 5% starch paste, drying, and sieving. Metformin and Linagliptin pulsatile beads were made by layering drug suspensions (with PVP binder and Aerosilglidant) onto MCC inert cores using a fluidized-bed coating technique, followed by drying and curing. The drug-loaded beads were seal-coated with HPMC K4M (factor A) and barrier-coated with Ethyl Cellulose EC7 CPS (factor B) and DOP (factor C) dissolved in IPA, following the factorial levels. After curing, the system was assembled into a pulse-in-cap design by filling size "0" hard gelatin capsules with three layers: immediate-release Metformin granules at the bottom, Metformin pulsatile beads in the middle, and Linagliptin pulsatile beads on top, achieving a total fill weight of 600 mg. Formulations were evaluated for particle size, entrapment efficiency, lag time, and in-vitro drug release to determine the optimized formulation. [25-31].

2.4 Evaluation of Pulsatile Drug Delivery System

2.4.1 Particle Size Analysis: Dynamic Light Scattering (DLS) or Laser Diffraction Particle Size Analyzer was used in measuring the particle size of the beads that were formulated and used in pulsatile drug delivery. About 10mg of beads was dispersed in distilled water and sonicated mildly to have a uniform suspension. Particle size measurement was done at 25°C in triple with the use of a clean cuvette in which the sample was transferred. The calibration of the instrument using a standard reference was carried out and then analyzed. Average mean diameter of the particles (Z-average) and polydispersivity index (PDI) was obtained to determine the homogeneity of the size distribution of the beads.

Table 1: Composition of formulations used for development of the ML-PULSE pulsatile drug delivery system

Ingredient	MLP1 (L,L,L)	MLP2 (H,L,L)	MLP3 (L,H,L)	MLP4 (H,H,L)	MLP5 (L,L,H)	MLP6 (H,L,H)	MLP7 (L,H,H)	MLP8 (H,H,H)
Immediate Release Part								
Metformin HCl (IR)	250	250	250	250	250	250	250	250
Soluble starch (5%)	27	27	26	26	26	26	27	25
Crosspovidone (1%)	10	10	10	10	10	10	10	10
Metformin Pulsatile Bead								
MCC bead	10	10	10	10	10	10	10	10
Metformin	250	250	250	250	250	250	250	250
PVP	3	2.5	3	2.5	2.5	2.5	3.5	1.5
Aerosil	10	8	11	11	9	6	10	10
Linagliptin Pulsatile Bead								
MCC bead	2	2	2	2	2	2	2	2
Linagliptin	5	5	5	5	5	5	5	5
PVP	3	2.5	3	2.5	2.5	2.5	3.5	1.5
Aerosil	10	8	10	10	9	6	10	10
Coating for both Beads								
Seal Coating (A factor) HPMC K4M	**4**	**8**	**4**	**8**	**4**	**8**	**4**	**8**
Coating Polymer (B factor) EC7 CPS	**5**	**5**	**10**	**10**	**5**	**5**	**10**	**10**
Plasticizer (C factor) DOP %	**6**	**6**	**6**	**6**	**12**	**12**	**12**	**12**
IPA	QS	QS	QS	QS	QS	QS	QS	QS
Total Capsule Fill (mg)	600	600	600	600	600	600	600	600

2.4.2 Entrapment Efficiency: Entrapment Efficiency (EE%) was determined to detect the percentage of Metformin or Linagliptin that was entrapped in the beads. About 100 mg of beads was either crushed or dissolved in phosphate buffer (pH 6.8) and centrifuged at 10000 rpm in 10 minutes. Supernatant was taken and examined under UV- Vis spectrophotometry at

232 nm (Metformin) or 299 nm (Linagliptin) in the framework of a calibration curve. The formula used to compute EE% was:

$$\text{Entrapment Efficiency (\%)} = (\text{Actual Drug Content in Beads}/\text{Theoretical Drug Content}) \times 100$$

2.4.3 In Vitro Lag Time, Drugs Release, and Release Kinetics:

Lag time was determined by placing pulsatile beads in pH 6.8 phosphate buffer at 37±0.5ºC and 100 rpm, with samples taken every 30 minutes and analyzed at 232 nm (Metformin) or 299 nm (Linagliptin). The lag time was defined as the point when 10% of the drug was released. A 12-hour release model was conducted using a USP Type II apparatus, with samples analyzed by UV or RP-HPLC. Drug release was modeled using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, with the optimal model selected based on R^2. Release mechanisms were classified using the Korsmeyer-Peppas model, aiding in the optimization of pulsatile release for Metformin and Linagliptin.

2.4.4Short-Term Accelerated Stability Study (ICH Q1A R2 Guidelines)

Short-term accelerated stability of optimized Metformin pulsatile beads was evaluated according to ICH Q1A(R2) guidelines as samples were stored in amber at 40 ± 2 °C / 75 ± 5% RH. At 0, 30, 60 and 90 days, particle size (DLS), entrapment efficiency, lag time, and 12 hours percentage drug release measurements were made as well as physical appearance and drug content. ANOVA was used to track the deviations to ensure that the beads were stable, release profile, and therapeutic efficacy at accelerated stress conditions.

2.4.5 *In Vivo* Pharmacokinetic Investigation of ADME Profiling

The pharmacokinetic in vivo experiment involved ADME profiling of the ADME ML-PULSE formulation or Linacent M 2.5/500 in the market against fasted male Wister rats in a randomized, two-treatment (two-treatment), two-period, two-sequence crossover study. The reference and test formulation (500 mg Metformin + 2. 5mg Linagliptin) were administered orally and plasma was sampled at the highest level of up to 24 hours. The concentrations of Metformin and Linagliptin were measured by use of HPLC with dual wavelength detection (232 nm and 299 nm) and important parameters were calculated, including Cmax, Tmax, AUC 0-t, AUC 0 -infinity, t 1/2, clearance (CL) and volume of distribution (Vd). The relative bioavailability was calculated based on the comparison of AUCs and statistical significance of differences among formulations was evaluated using either unpaired t -test or ANOVA ($p < 0.05$) to prove the superior pharmacokinetic profile of the pulsatile system.

3. RESULTS AND DISCUSSION

3.1 Drug and Excipient Compatibility Tests- DSC Analysis.

DSC analysis of Metformin and Linagliptin, along with their physical mixtures with excipients, showed high thermal stability and compatibility. Metformin exhibited a sharp melting point at 236.7ºC, with no major degradation or interaction with excipients (PVP, Aerosil, HPMC K4M, EC), confirmed by a similar peak at 238.45ºC in the physical mixture. This was an indicator of compatibility and suitability for controlled release formulations. A sharp endothermic peak was observed for linagliptin at 202.7ºC and a peak was observed for the physical mixture at 201.98ºC, which suggests that there were no adverse reactions with excipients. Both formulations were thermally stable and compatible which allows for the safe incorporation of excipients within pharmaceutical preparations without the loss of the thermal integrity of the drug.

Natural Resources for Human Health
eISSN: 2583-1194

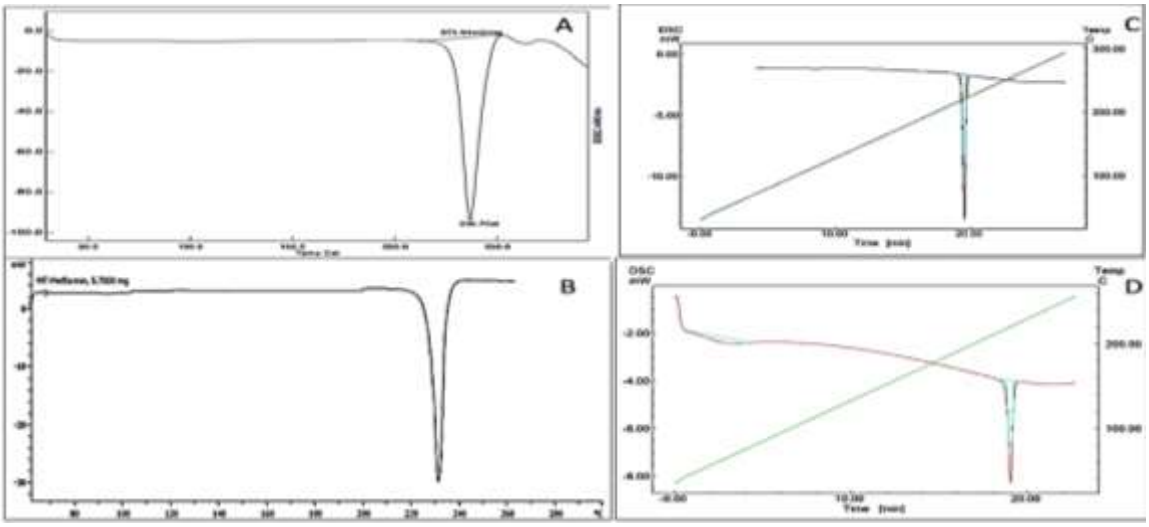

Figure 1: (A) DSC thermograms of Metformin, (B) Physical Mixture with metformin, (C) Linagliptin and (D) Physical Mixture of Linagliptin

3.2 Physicochemical Characteristics of Metformin HCl and Linagliptin

Metformin HCl is a white crystalline solid, highly polar, basic (pKa 12.4) hygroscopic solid with a high melting point (222-240°C). It is orally bioavailable and poorly penetrates tissues. Linagliptin, with a weak aqueous solubility (~0.9 mg/mL), is insoluble in lipophilic and aqueous solvents (log P 2.5, pKa 8.0) and also has a high melting point. All these properties require the use of moisture controlled and hydrophilic excipients to increase absorption and bioavailability.

3.3 Metformin HCl and Linagliptin UV-Vis Spectrophotometry

The UV-Vis method for Metformin HCl 232 nm exhibited very good linearity (R2 = 0.9996), thus confirming the Beer-Lambert law and demonstrating the precision for the quantification of formulations and biological samples. The method was also found to be highly linear and accurate (R^2 = 0.9915) for Linagliptin in the range of 2-10 µg/mL, with good sensitivity for low concentrations (2 µg/mL). Both methods were accurate, reliable and appropriate for analytical and routine quality control applications.

3.4 Evaluation of ML-PULSE Pulsatile Drug Delivery System

Effect of factors on Particle Size of Metformin Pulsatile pellet (MP):

The 2^3 factorial evaluation of ML-PULSE Metformin pulsatile pellets demonstrated that formulation variables HPMC K4M (A), Ethyl Cellulose (B), and DOP (C) significantly influenced particle size, entrapment efficiency, lag time, and drug release., The optimized formulation exhibited a mean particle size of approximately 1336 nm.with the regression model showing strong particle size reduction by A and B and a slight increase by C; the smallest size was achieved at low A–B and high C, corresponding to MP4. Entrapment efficiency ranged from 64.6% to 98.5%, and was mainly enhanced by high DOP and high HPMC K4M, while Ethyl Cellulose decreased EE, with maximum entrapment again observed in MP4. Lag time across formulations remained around 2.5–3.0 hours, with all factors showing only minor influence and MP4 achieving the desired ~3-hour delay for pulsatile action. Drug release analysis confirmed DOP as the strongest enhancer of release, while Ethyl Cellulose and the AC interaction reduced it; the highest 12-hour release occurred at low B and high C, matching MP4 conditions. Overall, MP4 emerged as the optimized formulation, providing the best balance of properties—smallest particle size (1336 nm), highest entrapment (98.5%), ideal lag time (3 h), and maximum sustained release (88.4%)—making it the most effective pulsatile delivery system.

Natural Resources for Human Health
eISSN: 2583-1194

Table 2: Optimization of Metformin Pellets (MP) pulsatile drug delivery system by 2^3 Factorial Design Final Factorial Table with Responses

Formulation	Particle Size (nm)	Entrapment Efficiency (%)	Lag Time (hr)	% Drug Release at 12hr
MLP1	**2814.9 ± 22.6**	**75.6 ± 2.4**	**3.0 ± 0.6**	**79.6 ± 2.8**
MLP2	**1336.0 ± 20.6**	**98.5 ± 2.8**	**3.0 ± 0.8**	**88.4 ± 2.8**
MLP3	**3378.4 ± 24.2**	**64.6 ± 3.2**	**2.5 ± 0.6**	**58.4 ± 2.4**
MLP4	**2823.3 ± 24.6**	**89.4 ± 2.6**	**2.5 ± 0.4**	**72.4 ± 2.6**
MLP5	**1836.1 ± 15.4**	**93.4 ± 2.6**	**3.0 ± 0.8**	**78.5 ± 2.8**
MLP6	**1929.4 ± 12.8**	**96.8 ± 2.2**	**2.5 ± 0.4**	**65.2 ± 2.6**
MLP7	**1634.0 ± 12.6**	**78.4 ± 3.8**	**3.0 ± 0.6**	**80.6 ± 2.6**
MLP8	**1765.1 ± 16.4**	**82.4 ± 2.6**	**3.0 ± 0.4**	**79.4 ± 2.8**
IR Metformin	**-**	**-**	**-**	**99.54 ±5.4 at 1.5 hr**

Particle Size Distribution and SEM Imaging of Metformin Pulsatile Beads MP 4

The DLS analysis (Figure 3A) revealed that the particle size was 1336 nm with PDI of 0.794 which represented a moderately broad size distribution. Despite having such a high PDI the single large peak at 570.4 nm with a 100% peak intensity indicated that, the majority of the beads were around a single size, with the occasional large aggregates increased the Z-average. SEM image (Figure 3B) was used to verify that the beads were spherical, smooth and without cracks, which were good encapsulators and mechanically stable. The fact that there was minor clustering of the image also contributed to the large value of PDI. In general, the morphology facilitated even coating and pulsatile release of equal intensity. The most suitable formulation was MP4 because it had the lowest particle size, had the highest entrapment efficiency, suitable lag time, and optimum drug release of 12 hours. It demonstrated the greatest potential in pulsatile delivery of drugs.

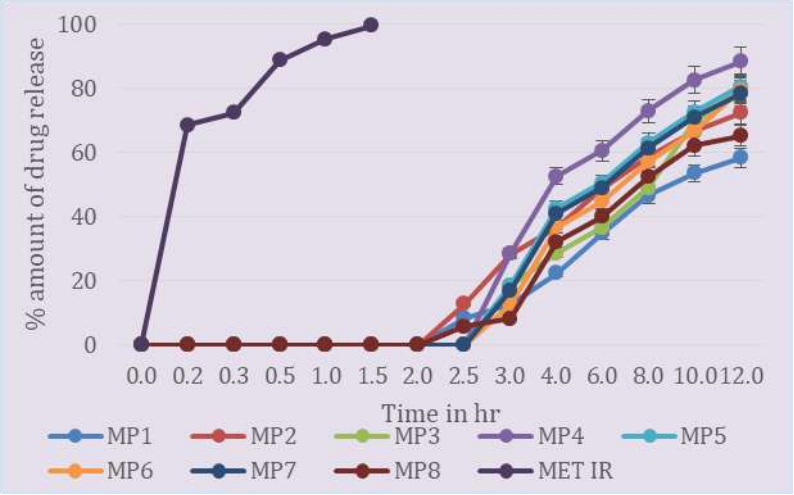

Figure 2: Invitro drug release studies showing %age amount of drug release for Meformin IR granules Vs Metformin Pulsatile pellets (MP)

Natural Resources for Human Health
eISSN: 2583-1194

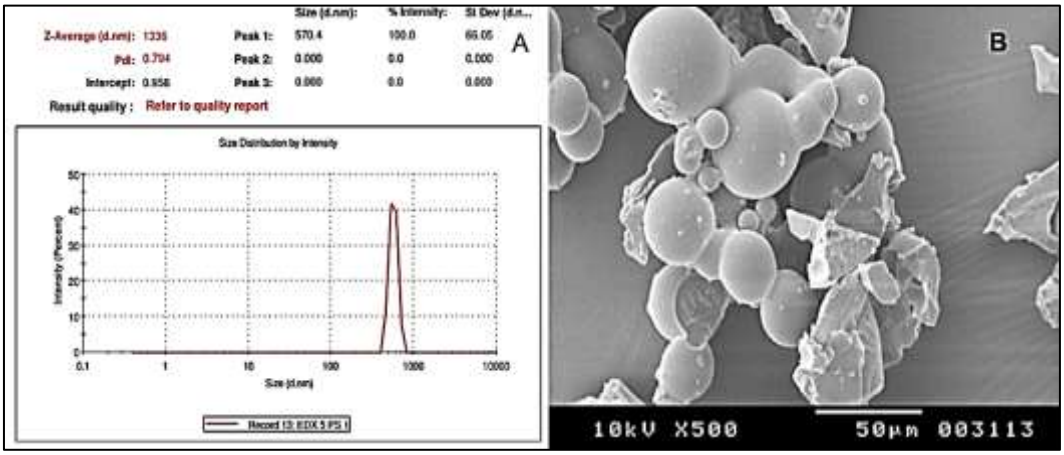

Figure 3: (A) Particle size and (B) SEM Images of Metformin Pulsatile Bead (MP)

3.5 Evaluation of Linagliptin Pulsatile Drug Delivery System

Effect of factors on Particle Size of Linagliptin Pulsatile pellet (LP):

The 2^3 factorial evaluation of Linagliptin pulsatile pellets (LP1–LP8) showed that formulation variables HPMC K4M (A), EC7 CPS (B), and DOP (C) strongly influenced particle size, entrapment efficiency, lag time, and drug release. Particle size ranged from 1634.0 nm (LP5) to 4336.8 nm (LP1), with regression analysis revealing that increasing HPMC and EC7 significantly reduced particle size, while higher DOP slightly increased it; the smallest size was consistently observed in LP5 (high A, high B, low C). Entrapment efficiency ranged from 72.5% to 92.4% and the AB interaction (HPMC × EC7) had the greatest positive effect along with further increase through HPMC alone, demonstrating that high A and B with low C resulted in highest entrapment, once again HPMC was best. Lag time was between 1.5 and 2.5 h and the model indicated that HPMC was the main factor in the increase in the lag time, while the effect of EC7, DOP and interactions were negligible; the lag time of LP5 was the longest, and the most controlled in all respect, which confirmed the predictable pulsatile behavior, confirming HPMC as the main factor. Drug release at 12 h was in the range of 56.6% (LP1) to 88.6% (LP5) and the factorials model showed a significant positive effect for HPMC and AB interaction, and weak or insignificant effect for EC7, DOP and higher order, with the highest release being found at high A + high B + low C (LP5). In all the responses tested – the smallest particle size, highest entrapment, optimal lag time and maximum sustained release, LP5 was found to be best and most optimized formulation for pulsatile delivery of Linagliptin.

Table 3: Optimisation of Linagliptin Pellets (LP) Pulsatile Drug Delivery system using 2^3 Factorial Design

Run	LP	A HPMC K4M	B EC7 CPS	C DOP %	Particle Size in nm	Entrapment Efficiency %	Lag Time in hr	Drug Release %
F1	LP3	4 mg	5 mg	1.00%	3040.9 ± 122.6	76.8 ± 6.4	2.0 ± 0.6	72.6 ± 3.4
F2	LP4	4 mg	5 mg	3.00%	1760.0 ± 122.8	86.4 ± 8.8	2.0 ± 0.8	82.4 ± 4.8
F3	LP1	4 mg	10 mg	1.00%	4336.8 ± 124.2	74.8 ± 8.4	1.5 ± 0.6	56.6 ± 4.4
F4	LP2	4 mg	10 mg	3.00%	3823.3 ± 106.8	72.6 ± 7.6	1.5 ± 0.4	64.4 ± 3.6
F5	LP7	8 mg	5 mg	1.00%	1836.1 ± 112.8	78.4 ± 5.7	2.5 ± 0.8	72.6 ± 4.4
F6	LP8	8 mg	5 mg	3.00%	1929.4 ± 105.8	72.5 ± 7.8	2.0 ± 0.4	65.2 ± 3.6
F7	LP5	8 mg	10 mg	1.00%	1634.0 ± 110.8	92.4 ± 5.8	2.5 ± 0.6	88.6 ± 3.6
F8	LP6	8 mg	10 mg	3.00%	1765.1 ± 118.4	83.5 ± 6.6	2.5 ± 0.4	78.4 ± 4.2

The optimized LP5 formulation showed a Z-average particle size of 1634 nm, consistent with factorial design predictions. The PDI was 1.000 which is generally considered to be good distribution, but revealed a single high peak at 220.2 nm, consistent with a uniform batch of beads with controlled bead formation. The tight PSD profile ensures the consistent particle size is maintained which is critical to the lag time and drug release in pulsatile systems. The SEM images (750×) showed that the beads were spherical and smooth, and had no defects in shape or structure, suggesting that the coating was successful and the beads had good mechanical strength. Minor surface deposits are probably excipient residues and are not likely to affect stability. The set of the data of both PSD and SEM validate the stability of LP5 size, morphology and coating. Among the formulations, LP5 showed the smallest particle size, highest entrapment efficiency, proper lag time and best drug release at 12 h and was thus considered to be the best formulation for pulsatile delivery of metformin.

Release Kinetics Data

The release kinetics of Metformin (MP4) and Linagliptin (LP5) were studied by applying different models which are Zero-order, First-order, Higuchi and Korsmeyer–Peppas.MP4 showed the best fit with the Zero-order model (R^2 = 0.9842), indicating constant, concentration-independent release, with a Korsmeyer–Peppas n-value of 0.581 confirming anomalous (diffusion–erosion) behavior. LP5 showed the strongest fit with the Korsmeyer–Peppas model (R^2 = 0.9800; n = 0.803), also indicating anomalous diffusion–erosion, and a strong Zero-order fit (R^2 = 0.9720). Both drugs had high Higuchi R^2 values, supporting diffusion-controlled release, while First-order kinetics showed poor correlation. Overall, MP4 followed Zero-order release, and LP5 followed anomalous diffusion-erosion with near Zero-order characteristics, demonstrating predictable and controlled pulsatile drug delivery.

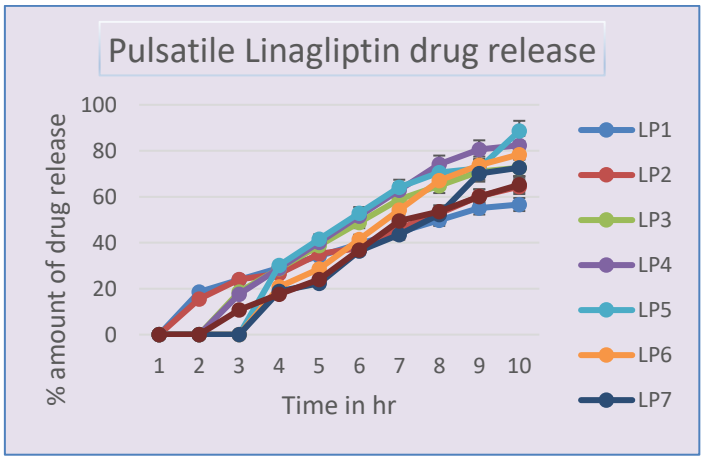

Figure 4: *Invitro* drug release studies showing %age amount of drug release for Linagliptin Pulsatile pellets (LP)

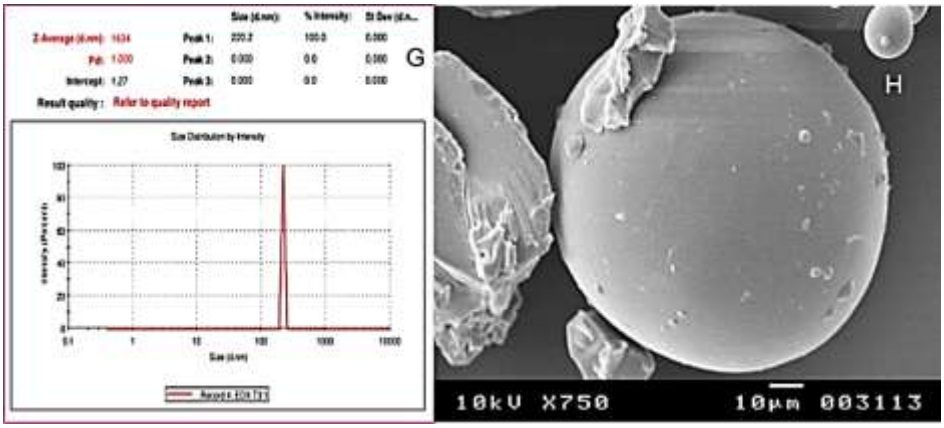

Figure 5: (G) Particle size and (H) SEM Images of Linagliptin Pulsatile Bead (LP)

Natural Resources for Human Health
eISSN: 2583-1194

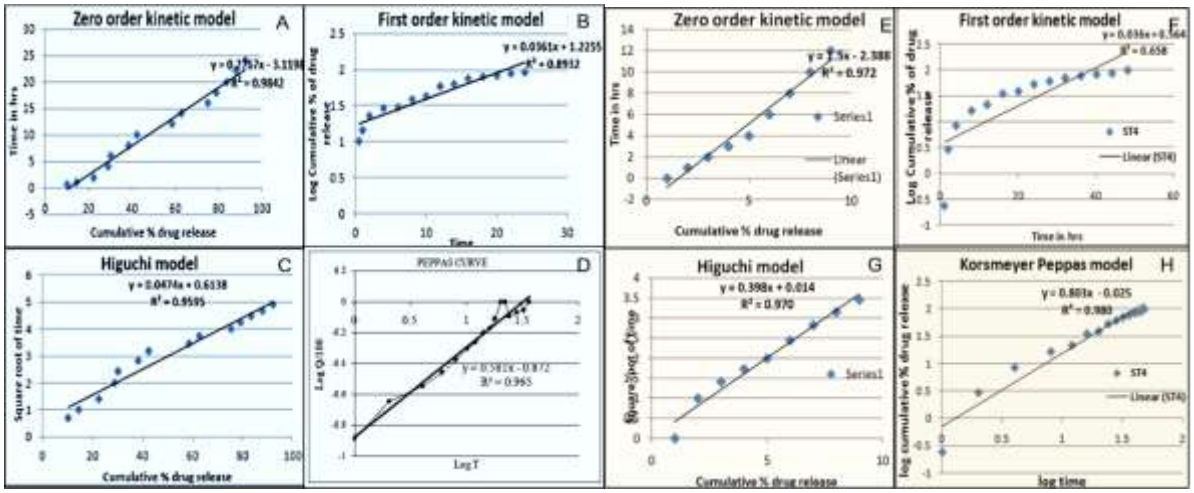

Figure 6: Release Kinetic Studies of Metformin Pulsatile pellets (A to D) & Release Kinetic Studies of Linagliptin Pulsatile pellets (E to H)

Accelerated Stability Studies (Short- and Long-Term):

All three formulations (MP4, LP5 and Met IR) showed good stability under conditions of accelerated ICH Q1A(R2) 40 ± 2 °C / 75 ± 5% RH /3 months). There was negligible changes in particle size and entrapment efficiency for MP4 while the lag time and 12 hour release remained almost the same which indicated the gradual preservation of its pulsatile mechanism. LP5 also exhibited slight increases in the particle size and small decreases in EE, and stable Lag time and 12-h release, suggesting stable pulsatile performance. The drug release was retained while maintaining rapid release characteristics with a slight decrease at 1.5 h in the case of Met IR. Overall, all formulations exhibited stability, were well suited for long-term therapeutic use and exhibited the intended release.

Table 4: Short-term Accelerated Stability Study for Metformin Pulsatile Beads (MP4) and Immediate Release Formulation (Met IR) - ICH Q1A (R2) guidelines

Parameter	Formulation	Initial (0 month)	1 month	2 months	3 months
Particle size (nm)	MP4	1336.0 ± 20.6	1342.5 ± 22.8	1350.1 ± 24.3	1360.7 ± 25.6
Entrapment Efficiency (%)	MP4	98.5 ± 2.8	97.8 ± 3.1	96.9 ± 3.4	96.1 ± 3.6
Lag time (hr)	MP4	3.0 ± 0.8	3.1 ± 0.9	3.2 ± 0.8	3.2 ± 0.9
% Drug release	MP4 (at 12 hr)	88.4 ± 2.8	87.9 ± 3.0	87.4 ± 3.3	87.0 ± 3.5
	Met IR (1.5 hr)	99.54 ± 5.4	98.95 ± 5.6	98.27 ± 5.8	97.64 ± 5.9

Table 5: Short-term Accelerated Stability Study for Linagliptin Pulsatile Beads (LP5) - ICH Q1A (R2) guidelines

Parameter	Initial (0 month)	1 month	2 months	3 months
Particle size (nm)	1634.0 ± 110.8	1645.5 ± 115.2	1658.3 ± 118.6	1672.4 ± 120.4
Entrapment Efficiency (%)	92.4 ± 5.8	91.8 ± 6.2	90.7 ± 6.5	89.6 ± 6.8
Lag time (hr)	2.5 ± 0.6	2.5 ± 0.7	2.6 ± 0.6	2.6 ± 0.7
% Drug release (12 hr)	88.6 ± 3.6	88.2 ± 3.8	87.7 ± 4.0	87.1 ± 4.2

Table 6: Long-term stability of Metformin Pulsatile Beads (MP4) and Linagliptin Pulsatile Beads (LP5) under ICH Q1A (R2) guidelines

Natural Resources for Human Health
eISSN: 2583-1194

Parameter	Formulation	0 month	3 months	6 months	9 months	12 months
Particle size (nm)	MP4	1336.0 ± 20.6	1340.8 ± 21.4	1348.3 ± 23.5	1355.7 ± 24.2	1363.5 ± 25.6
	LP5	1634.0 ± 110.8	1643.7 ± 112.4	1652.6 ± 114.3	1663.1 ± 116.2	1675.4 ± 118.7
Entrapment Efficiency (%)	MP4	98.5 ± 2.8	97.9 ± 3.0	97.3 ± 3.2	96.8 ± 3.4	96.3 ± 3.6
	LP5	92.4 ± 5.8	91.6 ± 5.9	90.9 ± 6.2	90.2 ± 6.4	89.8 ± 6.5
Lag time (hr)	MP4	3.0 ± 0.8	3.0 ± 0.8	3.1 ± 0.7	3.1 ± 0.8	3.2 ± 0.8
	LP5	2.5 ± 0.6	2.5 ± 0.6	2.6 ± 0.7	2.6 ± 0.7	2.6 ± 0.7
% Drug release (12 hr)	MP4	88.4 ± 2.8	88.1 ± 3.0	87.7 ± 3.2	87.4 ± 3.4	87.0 ± 3.5
	LP5	88.6 ± 3.6	88.3 ± 3.7	87.9 ± 3.9	87.5 ± 4.1	87.2 ± 4.2

3.6 *In vivo* Pharmacokinetic studies

Table 7: Invivo Pharmacokinetic Data of ML-Pulse Pulsatile drug delivery system

Time (hr)	Metformin IR Mean±SD	MP4 Mean±SD	LP5 Mean±SD	Marketed Mean±SD
0	0 ±0	0±0	0±0	0±0
0.5	25.02 ±2.02	0±0	0±0	20.24 ±2
1	50.12±5.02	0±0	0±0	40.12±3.5
2	30.04±3.12	0±0	0±0	38.24 ±3
4	20.12±2.02	40.12±3.20	35.12±3.12	35.12±2.8
6	12.02±1.50	45.22±2.82	40.24±2.62	25.22±2
8	8.02±1.12	42.08±2.52	38.32±2.40	15.34±1.5
10	4.00±0.08	38.14±2.24	34.18±2.21	5.10±1
12	2.02±0.05	30.12±2.02	28.22±1.08	2.16±0.5

Table 8: Pharmacokinetic Data: ML-PULSE vs Marketed and IR Formulations

Parameter	Metformin IR	MP4 (Metformin PDDS)	LP5 (LinagliptinPDDS)	Marketed (LinacentM)
Cmax (ng/mL)	50.12	45.22	40.24	40.12
Tmax (hr)	1.0	6.0	6.0	1.0
AUC_{0-t} (ng·hr/mL)	185.50	361.24	323.94	261.29
$AUC_{0-\infty}$(ng·hr/mL)	191.36	721.55	692.90	265.70
$t_{1/2}$ (hr)	2.01	8.29	9.06	1.41

K_{el}(1/hr)	0.3447	0.0836	0.0765	0.4901
CL (L/hr)	0.52	0.14	0.14	0.38
V_d (L)	1.52	1.66	1.89	0.77

MP4 and LP5 show significantly improved pharmacokinetic performance over Metformin IR and the marketed formulation, nearly doubling bioavailability (AUC_0–t: MP4 = 361.24, LP5 = 323.94 vs. IR = 185.50) and prolonging systemic exposure (AUC_0–∞: MP4 = 721.55, LP5 = 692.90). The formulations have a Tmax of 6 hours which correspond to the circadian glucose peak in diabetics, and long half-lives of MP4 (8.29 hours) and LP5 (9.06 hours). Lower elimination rates (Kel = 0.0836 and 0.0765) and lower clearance (~0.14 L/hr) result in steady circulation and less peak to trough fluctuation. Additionally, higher volumes of distribution (MP4: 1.66 L, LP5: 1.89 L) enable better tissue penetration and prolonged therapeutic action. The marketed formulation and Metformin IR have no chronotherapeutic advantage, are highly peaks in 1 hour and are used for immediate glycemic control. MP4 and LP5 are optimized, chronotherapy-oriented pulsatile formulations that provide better glucose rhythm control, improved bioavailability and longer action. The ML-PULSE system (MP4 and LP5) is more bioavailable and synchronizes the circadian rhythm than Metformin IR and marketed products. The bioavailability is almost twice as high with MP4 and LP5 (2× AUC of IR) and the release is delayed for 3 hours, matching the peak in glucose concentrations in the early morning. The marketed formulation, however, is devoid of sustained control and rhythmic adaptation, as are Metformin IR. This ML-PULSE system enhances drug bioavailability, prolongs the effect of the drug and decreases fluctuations, resulting in increased patient compliance. It has an extended Tmax (6 hours) and half-life, which may contribute to improved glycemic control and insulin sensitivity. Its stability and predictable profile make it an attractive option for developing new therapies for T2DM.

4. CONCLUSION

The ML-PULSE pulsatile drug delivery system is a big step forward in type 2 diabetes treatment, with enhanced bioavailability and circadian controlled drug release. The optimized MP4 (Metformin) and LP5 (Linagliptin) formulations exhibited superior pharmacokinetics, delayed release and prolonged therapeutic activity than the traditional and commercial products. By releasing drugs in response to the glucose spike in the morning, lowering the dose frequency and increasing patient adherence, ML-PULSE is a leading chronotherapeutic platform. Overall, MP4 and LP5 are promising platforms for clinical use and could be modified for other diseases that require time-programmed and sustained drug delivery.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

Ravali V: Conceptualization, methodology, formulation development, experimental investigation, data collection, data analysis, interpretation of results, and manuscript preparation.

Balaji P: Supervision, guidance in study design and methodology, review and interpretation of results, critical revision of the manuscript, and overall supervision of the research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

The animal study was approved by the Institutional Animal Ethics Committee (IAEC) of Sri Lakshmi Narayana Institute of Medical Sciences & Hospital, Pondicherry, under Meeting Ref. No. **03/SLIMS/IAEC/2025-26** and Proposal No. **SLIMS/03-09/IAEC/2025-26**, dated **03 June 2025**.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

[1] Tekade AR, Gattani SG. Development and evaluation of pulsatile drug delivery system using novel polymer. Pharm Dev Technol. 2009;14(4):380-7. doi: 10.1080/10837450802712625

[2] Pandey R, Selvamurthy W. Design and in vitro characterization of novel pulsatile delivery system of biguanideantidiabetic drug. J Pharm Bioallied Sci. 2020 Jul-Sep;12(3):356-368. doi: 10.4103/jpbs.JPBS_203_19.

[3] Hu LD, Liu Y, Tang X, Zhang Q. Preparation and in vitro/in vivo evaluation of sustained-release metformin hydrochloride pellets. Eur J Pharm Biopharm. 2006 Oct;64(2):185-92. doi: 10.1016/j.ejpb.2006.04.004.

[4] Tiwari R, Gupta A, Joshi M, Tiwari G. Bilayer Tablet Formulation of Metformin HCl and Acarbose: A Novel Approach to Control Diabetes. PDA J Pharm Sci Technol. 2014; 68(2):138-52. doi: 10.5731/pdajpst.2014.00953.

[5] Gioumouxouzis CI, Baklavaridis A, Katsamenis OL, Markopoulou CK, Bouropoulos N, Tzetzis D, Fatouros DG. A 3D printed bilayer oral solid dosage form combining metformin for prolonged and glimepiride for immediate drug delivery. Eur J Pharm Sci. 2018 Jul 30; 120:40-52. doi: 10.1016/j.ejps.2018.04.020.

[6] vanHaaren C, De Bock M, Kazarian SG. Advances in ATR-FTIR Spectroscopic Imaging for the Analysis of Tablet Dissolution and Drug Release. Molecules. 2023 Jun 12;28(12):4705. doi: 10.3390/molecules28124705.

[7] Kazarian SG, Ewing AV. Applications of Fourier transform infrared spectroscopic imaging to tablet dissolution and drug release. Expert Opin Drug Deliv. 2013 Sep;10(9):1207-21. doi: 10.1517/17425247.2013.801452. Wray P, Li J, Li LQ, Kazarian SG. Combined study of biphasic and zero-order release formulations with dissolution tests and ATR-FTIR spectroscopic imaging. J Pharm Sci. 2014 Jul;103(7):1995-2004. doi: 10.1002/jps.23987.

[8] Zahoor FD, Mader KT, Timmins P, Brown J, Sammon C. Investigation of Within-Tablet Dynamics for Extended Release of a Poorly Soluble Basic Drug from Hydrophilic Matrix Tablets Using ATR-FTIR Imaging. Mol Pharm. 2020 Apr 6;17(4):1090-1099. doi: 10.1021/acs.molpharmaceut.9b01063.

[9] Mendes TC, Simon A, Menezes JCV, Pinto EC, Cabral LM, de Sousa VP. Development of USP Apparatus 3 Dissolution Method with IVIVC for Extended-Release Tablets of Metformin Hydrochloride and Development of a Generic Formulation. Chem Pharm Bull (Tokyo). 2019;67(1):23-31. doi: 10.1248/cpb.c18-00579.

[10] Sokar M, Hanafy A, Elkamel A, El-Gamal S. Design of Chronomodulated Drug Delivery System of Valsartan: In Vitro Characterization. Indian J Pharm Sci. 2015 Jul-Aug;77(4):470-7. doi: 10.4103/0250-474x.164768.

[11] Lin HL, Lin SY, Lin YK, HoHo, Lo YW, Sheu MT. Release characteristics and in vitro-in vivo correlation of pulsatile pattern for a pulsatile drug delivery system activated by membrane rupture via osmotic pressure and swelling. Eur J Pharm Biopharm. 2008 Sep;70(1):289-301. doi: 10.1016/j.ejpb.2008.03.021.

[12] Lin KH, Lin SY, Li MJ. Compression forces and amount of outer coating layer affecting the time-controlled disintegration of the compression-coated tablets prepared by direct compression with micronized ethylcellulose. J Pharm Sci. 2001 Dec;90(12):2005-9. doi: 10.1002/jps.

[13] Lin SY, Li MJ, Lin KH. Hydrophilic excipients modulate the time lag of time-controlled disintegrating press-coated tablets. AAPS PharmSciTech. 2004 Aug 16;5(4): e54. doi: 10.1208/pt050454.

[14] Lin SY, Lin KH, Li MJ. Formulation design of double-layer in the outer shell of dry-coated tablet to modulate lag time and time-controlled dissolution function: studies on micronized ethylcellulose for dosage form design (VII). AAPS J. 2004 Jul 14;6(3): e17. doi: 10.1208/aapsj060317.

[15] Paolisso G, Scheen AJ, Giugliano D, Sgambato S, Albert A, Varricchio M, D'Onofrio F, Lefèbvre PJ. Pulsatile insulin delivery has greater metabolic effects than continuous hormone administration in man: importance of pulse frequency. J ClinEndocrinolMetab. 1991 Mar;72(3):607-15. doi: 10.1210/jcem-72-3-607.

[16] Lin SY, Lin KH, Li MJ. Micronized ethylcellulose used for designing a directly compressed time-controlled disintegration tablet. J Control Release. 2001 Feb 23;70(3):321-8. doi: 10.1016/s0168-3659(00)00360-6.

[17] Lin SY, Lin KH, Li MJ. Formulation design of double-layer in the outer shell of dry-coated tablet to modulate lag time and time-controlled dissolution function: studies on micronized ethylcellulose for dosage form design (VII). AAPS J. 2004 Jul 14;6(3): e17. doi: 10.1208/aapsj060317.

[18] Lin SY, Li MJ, Lin KH. Hydrophilic excipients modulate the time lag of time-controlled disintegrating press-coated tablets. AAPS PharmSciTech. 2004 Aug 16;5(4): e54. doi: 10.1208/pt050454.

[19] Watanabe Y, Mukai B, Kawamura K, Ishikawa T, Namiki M, Utoguchi N, Fujii M. [Preparation and evaluation of press-coated aminophylline tablet using crystalline cellulose and polyethylene glycol in the outer shell for timed-release dosage forms]. YakugakuZasshi. 2002 Feb;122(2):157-62. Japanese. doi: 10.1248/yakushi.

[20] Fukui E, Miyamura N, Uemura K, Kobayashi M. Preparation of enteric coated timed-release press-coated tablets and evaluation of their function by in vitro and in vivo tests for colon targeting. Int J Pharm. 2000 Aug 25;204(1-2):7-15. doi: 10.1016/s0378-5173(00)00454-3.
[21] Jain D, Raturi R, Jain V, Bansal P, Singh R. Recent technologies in pulsatile drug delivery systems. Biomatter. 2011 Jul-Sep;1(1):57-65. doi: 10.4161/biom.1.1.17717.
[22] Sawada T, Kondo H, Nakashima H, Sako K, Hayashi M. Time-release compression-coated core tablet containing nifedipine for chronopharmacotherapy. Int J Pharm. 2004 Aug 6;280(1-2):103-11. doi: 10.1016/j.ijpharm.2004.05.004.
[23] Shah S, Patel R, Soniwala M, Chavda J. Development and optimization of press coated tablets of release engineered valsartan for pulsatile delivery. Drug DevInd Pharm. 2015;41(11):1835-46. doi: 10.3109/03639045.2015.1014374.
[24] Qureshi J, Ahuja A, Baboota S, Chutani K, Jain S, Ali J. Development and evaluation of a time-specific pulsatile-release tablet of aceclofenac: a solution for morning pain in rheumatoid arthritis. Methods Find ExpClinPharmacol. 2009 Jan-Feb;31(1):15-23. doi: 10.1358/mf.2009.31.1.1338412.
[25] Rajput A, Pingale P, Telange D, Musale S, Chalikwar S. A current era in pulsatile drug delivery system: Drug journey based on chronobiology. Heliyon. 2024 Apr 4;10(10): e29064. doi: 10.1016/j.heliyon.2024.e29064.
[26] Chaudhari PM, Chaudhari PD. Formulation and evaluation of multiparticulate system for chronotherapeutic delivery of salbutamol sulphate. J Pharm Bioallied Sci. 2012 Mar;4(Suppl 1): S71-3. doi: 10.4103/0975-7406.94144.
[27] Battu S, Yalavarthi PR, Gopireddy VSR, Vattikuti UMR, Devi J, Vadlamudi HC. Chronopharmacokinetic Evaluation of Budesonide Multiparticulate Systems. Recent Pat Drug DelivFormul. 2017;11(3):221-229. doi: 10.2174/1872211312666171213113127.
[28] Yadav D, Survase S, Kumar N. Dual coating of swellable and rupturable polymers on glipizide loaded MCC pellets for pulsatile delivery: formulation design and in vitro evaluation. Int J Pharm. 2011 Oct 31;419(1-2):121-30. doi: 10.1016/j.ijpharm.2011.07.026.
[29] Handattu MS, Thirumaleshwar S, Prakash GM, Somareddy HK, Veerabhadrappa GH. A Comprehensive Review on Pellets as a Dosage Form in Pharmaceuticals. Curr Drug Targets. 2021;22(10):1183-1195. doi: 10.2174/1389450122999210120204248.
[30] Roy P, Shahiwala A. Multiparticulate formulation approach to pulsatile drug delivery: current perspectives. J Control Release. 2009 Mar 4;134(2):74-80. doi: 10.1016/j.jconrel.2008.11.011.
[31] Gazzaniga A, Palugan L, Foppoli A, Sangalli ME. Oral pulsatile delivery systems based on swellable hydrophilic polymers. Eur J Pharm Biopharm. 2008 Jan;68(1):11-8. doi: 10.1016/j.ejpb.2007.05.022.
[32] Gandhi BR, Mundada AS, Gandhi PP. Chronopharmaceutics: as a clinically relevant drug delivery system. Drug Deliv. 2011 Jan;18(1):1-18. doi: 10.3109/10717544.2010.509358. PMID: 21138394.
[33] Maroni A, Zema L, Cerea M, Sangalli ME. Oral pulsatile drug delivery systems. Expert Opin Drug Deliv. 2005 Sep;2(5):855-71. doi: 10.1517/17425247.2.5.855. PMID: 16296783.
[34] Bussemer T, Otto I, Bodmeier R. Pulsatile drug-delivery systems. Crit Rev Ther Drug Carrier Syst. 2001;18(5):433-58.
[35] Patil SS, Shahiwala A. Patented pulsatile drug delivery technologies for chronotherapy. Expert OpinTher Pat. 2014 Aug;24(8):845-56. doi: 10.1517/13543776.2014.916281.
[36] Mandal AS, Biswas N, Karim KM, Guha A, Chatterjee S, Behera M, Kuotsu K. Drug delivery system based on chronobiology--A review. J Control Release. 2010 Nov 1;147(3):314-25. doi: 10.1016/j.jconrel.2010.07.122.
[37] Politis SN, Rekkas DM. Recent advances in pulsatile oral drug delivery systems. Recent Pat Drug DelivFormul. 2013 Aug;7(2):87-98. doi: 10.2174/1872211311307020001.
[38] Maroni A, Zema L, Del Curto MD, Loreti G, Gazzaniga A. Oral pulsatile delivery: rationale and chronopharmaceutical formulations. Int J Pharm. 2010 Oct 15;398(1-2):1-8. doi: 10.1016/j.ijpharm.2010.07.026.
[39] Maroni A, Zema L, Loreti G, Palugan L, Gazzaniga A. Film coatings for oral pulsatile release. Int J Pharm. 2013 Dec 5;457(2):362-71. doi: 10.1016/j.ijpharm.2013.03.010.
[40] Lin SY, Kawashima Y. Current status and approaches to developing press-coated chronodelivery drug systems. J Control Release. 2012 Feb 10;157(3):331-53. doi: 10.1016/j.jconrel.2011.09.065.
[41] Siepmann F, Siepmann J, Walther M, MacRae RJ, Bodmeier R. Polymer blends for controlled release coatings. J Control Release. 2008 Jan 4;125(1):1-15. doi: 10.1016/j.jconrel.2007.09.012.
[42] Rujivipat S, Bodmeier R. Modified release from hydroxypropyl methylcellulose compression-coated tablets. Int J Pharm. 2010 Dec 15;402(1-2):72-7. doi: 10.1016/j.ijpharm.2010.09.021.

[43] Tran PH, Choe JS, Tran TT, Park YM, Lee BJ. Design and mechanism of on-off pulsed drug release using nonenteric polymeric systems via pH modulation. AAPS PharmSciTech. 2011 Mar;12(1):46-55. doi: 10.1208/s12249-010-9562-1.
[44] Pandey R, Selvamurthy W. Design and in vitro characterization of novel pulsatile delivery system of biguanideantidiabetic drug. J Pharm Bioallied Sci. 2020 Jul-Sep;12(3):356-368. doi: 10.4103/jpbs.JPBS_203_19.
[45] Jain D, Raturi R, Jain V, Bansal P, Singh R. Recent technologies in pulsatile drug delivery systems. Biomatter. 2011 Jul-Sep;1(1):57-65. doi: 10.4161/biom.1.1.17717.
[46] Till Bussemer, Ina Otto, Roland Bodmeier. Pulsatile Drug-Delivery Systems, CritRevTherDrugCarrierSyst.18 (5), 2001, 26. DOI: 10.1615/ v18.i5.10
[47] Rajput A, Pingale P, Telange D, Musale S, Chalikwar S. A current era in pulsatile drug delivery system: Drug journey based on chronobiology. Heliyon. 2024 Apr 4;10(10): e29064. doi: 10.1016/j.heliyon.2024.e29064.
[48] Singh S, Koland M. Formulation and evaluation of pulsatile drug delivery systems of glipizide for the management of type-ii diabetes mellitus. J. Drug Delivery Ther. [Internet]. 2016 Jan. 15;6(1):11-8.
[49] Ezike TC, Okpala US, Onoja UL, Nwike CP, Ezeako EC, Okpara OJ, Okoroafor CC, Eze SC, Kalu OL, Odoh EC, Nwadike UG, Ogbodo JO, Umeh BU, Ossai EC, Nwanguma BC. Advances in drug delivery systems, challenges and future directions. Heliyon. 2023 Jun 24;9(6): e17488. doi: 10.1016/j.heliyon.2023.e17488.