**Design and In Vitro Characterization of Novel Pulsatile Delivery System of Biguanide Antidiabetic Drug**

Rahul Pandey, William Selvamurthy

**Aim:** This work shows the development of pulsatile capsular pellets of metformin hydrochloride and its characterization. **Material and Methods:** The novel drug delivery system consisted of hydroxy propyl cellulose Type H. It has a lag time modifier and Eudragit L-100 and Eudragit S-100 in different concentrations as pH-dependent release modifier in the gastrointestinal tract. The pellets were subjected to in vitro release studies using USP dissolution apparatus type-II in distilled water, phosphate buffer of pH 6.8, and 0.1 N HCl and methanol. Ultraviolet (UV), infrared (IR), high-performance liquid chromatography (HPLC), and mass spectroscopy were performed for active pharmaceutical ingredients and formulations. **Result:** The study was characterized by the complete release of the drug in pulses after a well-defined lag time of 6 h (±0.20) (period of no drug release) for the treatment of type II diabetes mellitus. **Conclusion:** The stability studies on the selected formulation of Metformin were found to be stable, with shelf life of 1.94 years. Hence it may be concluded that the newly formulated pulsatile drug delivery systems of Metformin Hydrochloride, when ingested at the bed time in the night, produce effective control of the increased blood glucose level after intake of meals by allowing the drug to release after a lag time of 6 h (after meals).

**KEYWORDS:** Infrared, mass spectra, metformin hydrochloride, pellets, pulsatile

---

**INTRODUCTION**

Diabetes has become an epidemic. In 2017, according to the International Diabetes Federation, an estimated 451 million people had diabetes. Its prevalence is increasing rapidly, and by 2030, this number is estimated to be almost double.[1] There are many conventional dosage forms available in the market to treat the condition of diabetes. This study emphasizes on treating diabetes based on circadian rhythm. Hence, the pulsatile type of delivery system was designed and was characterized. The terminology pulsatile consists of the word “Pulse,” indicating a rhythmic beat. The pulsatile system is often misunderstood as a chronotherapeutic system. It comprises the release of drug after a definite lag time followed by abrupt or prompt release. Pulsatile systems are gaining a lot of interest as they deliver the drug at the right site of action at the right time, thus providing spatial and temporal delivery and increasing patient compliance. These systems are designed according to the circadian rhythm of the body. The circadian rhythm regulates many body functions in humans, namely metabolism, physiology, behavior, sleep patterns, and hormone production. It has been reported that more shocks and heart attacks occur during morning hours. The patients with diabetes are reported to have high blood sugar levels after meals compared to other timings. Almost all chronotherapeutic systems intended for treating the
conditions following the circadian rhythms release the drug after a lag phase in one single attempt. There is no single system developed for such conditions, which can release the drug in multiple pulses unless the formulation is intended to be taken more than once daily.[2]

These systems are beneficial for drugs having a high first-pass effect, drugs administered for diseases that follow chronopharmaceutical behavior, drugs having a specific absorption site in gastrointestinal tract (GIT), targeting to the colon, and cases where nighttime dosing is required. Chronotherapy targets the medication administered at the time when they are required the most.[3]

Metformin hydrochloride is widely used for the treatment of Type-2 diabetes mellitus. It is a biguanide developed from galegine, this guanidine derivative is found in Galega officinalis.[3] Chemically, metformin hydrochloride is a hydrophilic base; however, it is usually present in oral dosage forms in its hydrochloride salt form. Metformin hydrochloride has acid dissociation constant values (pKa) of 2.8 and 11.5, and therefore exists very large as the hydrophilic cationic species at physiological pH values (>99.9%).[3] This chemical parameter indicates low lipophilicity, and therefore rapid passive diffusion of metformin through cell membranes is not prevalent.[3] The lipid solubility of the unionized species is low as shown by its low water–oil partition coefficient value (logP = 1.43).[4] On the basis of these properties, metformin hydrochloride is defined as class III (low permeability, high solubility) by the Biopharmaceutics Classification System (BCS).[5]

The reason for the selection of metformin hydrochloride is that it has an extensive first-pass metabolism, develops biological tolerance, and exhibits poor bioavailability and erratic absorption.

The pulsatile drug delivery of metformin hydrochloride will overcome first-pass metabolism in liver as the drug is absorbed in the GIT. The pulsatile drug delivery system will overcome the biological tolerance of the drug. The bioavailability of the drug is improved with adequate absorption at the GIT.

Pulsatile drug release system allows the release of active pharmaceutical material in single or successive pulses at precise and well-controlled time. Assuming that physiological processes and biological functions display constancy over time, much effort had been done in the past in developing the drug delivery systems that maintain a flatter plasma level for an extended period.

The major objectives of the study include design and characterization of capsular pellets–based pulsatile drug delivery system for the treatment of diabetes mellitus with the lag time of 6h (±0.20) and perform in vitro studies and stability studies of the optimized formulation.

**Materials and Methods**

**Chemicals and reagents**

Metformin hydrochloride was obtained as a gift sample from Sun Pharma, Gurugram, India. Hydroxy propyl cellulose (HPC) Type H, Eudragit L-100, and Eudragit S-100 from Jubilant Generics Limited, Noida, India; sodium hydroxide, methanol (high-performance liquid chromatography [HPLC] Grade), and ethanol (95%) from Merck India, Mumbai, India; sodium chloride, triethyl citrate, and ethyl cellulose from S.D. Fine Chemicals, New Delhi, India. All the chemicals used in this study were of analytical grade.

**Preformulation study**

**Characterization of drug**

Physical description/organoleptic properties: The organoleptic properties refer to the appearance, color, odor, and taste of the substance. Characterization of these properties is the primary step in the preformulation study and helps with the primary identification of the drug substance and in the determination of the likely patient acceptability of the odor, taste, and color of the raw material and the possible inclusion in the final dosage form.

**Melting point**

Melting point of the drug was determined by taking a small amount of drug in a capillary tube closed at one end and was placed in Thiele’s melting point apparatus (Flinn Scientific Canada Inc., Hamilton, Canada). The temperature at which the drug melted was reported.

**Differential scanning calorimetry**

The physical state and melting point of the drug was determined by differential scanning calorimetry (DSC) (Perkin Elmer, Rostock, Germany). Samples containing 3 mg of drug was placed in pan in the instrument and heated from 50°C to 250°C, at a heating rate of 10°C/min, under inert atmosphere flushed with nitrogen at the rate of 20mL/min. Alumina was used as the reference standard. The onsets of melting points and enthalpies of fusion of samples were calculated by the instrument.[6]

**Particle size distribution by sieve method**

The sieves were weighed separately before the beginning of the experiment. They were arranged in ascending order of their mesh size (ASTM No.), that is, 30, 40,
Flow properties of metformin hydrochloride powder

Flowability

It is the term used to describe the flow properties of the solid particles. Uniform and reproducible feeding of the powder or granules from the hopper into the extrusion and spheronizer was done to achieve weight uniformity of the product.[7]

Angle of repose

The angle of repose is a simple practical measurement for indicating the flow of (particles). It is found to be the angle between the free-standing surface of powder heap and horizontal plane and is given by the following equation:

\[ \tan \theta = \frac{h}{r} \]  

(1)

Where \( \tan \theta \) = tangent of angle, \( r \) = radius of the heap (cm), and \( h \) = height of the heap (cm). Thus, the angle of repose is independent of the mass of powder. The fix base cone method was used to determine the angle of repose in this study.[8] In this method, the cone was kept in an upright position on a piece of white printing paper and filled with the test powder. Then the filled cone was smoothly and gently lifted allowing the flow of powder on the paper. The heap of powder was collected on a paper and the radius and height of the heap of powder was measured and angle of repose was calculated using the aforementioned formula.

Density studies

This is another property, which improves the characterization of the flow of powders. The tapped density of the powder provides a relationship between the degree of compaction and the flow properties.  

Bulk density

Approximately 30 g of metformin hydrochloride was weighed accurately and transferred into 100 mL capacity cylinder. After settling the powder, the volume was measured, which came out to be 62 mL. The bulk density was determined by the following formula:

\[ \text{Bulk density} = \frac{M}{V} \]  

(2)

Where \( M \) = mass of the sample and \( V \) = unsettled apparent volume.

Tapped density

Approximately 30 g of the drug was weighed accurately and transferred into 100 mL cylinder. Cylinder was tapped mechanically by raising the cylinder. It is allowed to drop under its own weight with fixed drop of 14 ± 2 mm at a normal rate of 300 drops per minute. The cylinder was tapped 500, 750, and 1250 times initially, and tapped volumes were measured.

\[ \text{Tapped density} = \frac{M}{V_f} \]  

(3)

where \( M \) = mass of the test sample and \( V_f \) = final tapped volume.

Powder compressibility

The compressibility index and the Hausner ratio are the measures of porosity of a powder to be compressed. They are calculated by the following equations:

Compressibility index:

\[ \text{Carr’s Compressibility Index} = \frac{100 (V_o - V_f)}{V_o} \]  

(4)

where \( V_o \) = unsettled apparent volume (under bulk density determination) and \( V_f \) = final tapped volume.

Hausner ratio:

\[ \text{Hausner’s ratio} = \frac{V_o}{V_f} \]  

(5)

where \( V_o \) = unsettled apparent volume and \( V_f \) = final tapped volume.

pH solubility studies

The pH solubility in distilled water with 100 ppm stock solution was prepared by dissolving 10 mg drug in 100 mL of vehicle, and then it was sonicated for 30 min.

Identification: metformin hydrochloride analysis

Ultraviolet spectral analysis

The stock solutions of drug in different media were scanned for absorbance in the region of 400–200 nm and ultraviolet (UV) absorption spectra were obtained using UV spectrophotometer (Shimadzu, Kitakyushu, Japan).

| Table 1: Particle size distribution by sieve analysis |
|---------------------------------------------------|
| **Sieve size** | **% Retained ± SD** |
| # 30 | 0 |
| # 40 | 0 |
| # 60 | 5.66 ± 11.4 |
| # 85 | 18.1 ± 12.5 |
| # 100 | 23.76 ± 10.5 |
| # 150 | 32.2 ± 11.6 |
| Pan | 19.8 ± 12.1 |
Fourier transform infrared spectroscopy

The infrared (IR) spectrum of drug was taken by using KBr pellet method.

Mass spectroscopy

The mass spectrometer was operated in positive ion selected reaction monitoring (SRM) mode. Metformin hydrochloride was monitored at a parent mass of 130.097 and a daughter mass of 71.14 with a tube lens voltage of 54.56 V and a collision energy of 22 V. The internal standard for phenformin was monitored at a parent mass of 206.167 and a daughter mass of 105.08, the tube lens and collision energy were 58.07 and 105.08 V, respectively. The capillary temperature was set at 270°C, collision pressure at 1.5 mTorr. The mass spectrometer software used for data capture was Xcalibur 2.0.7 and QuickQuan 2.3 (Thermo Fisher Scientific, San Jose, California).[9]

High-performance liquid chromatography

A stock solution of metformin hydrochloride having 1 mg/mL was made by dissolving 100 mg drug in 100 mL of mobile phase, RPC18 (LiChroCART, 250–4 i.d., 5 μ particle). Flow rate of mobile phase was 1.3 mL/min and injection volume 20 μL. Elute was analyzed at 236 nm. The mobile phase consisted of 2.4008 g sodium chloride in 1.7009 g of pentane sulfonate, dissolved in 3.5 mL with orthophosphoric acid.[10] 200 mL of water mixed well. The pH was adjusted to pH 6.8, 0.1 N HCl, and methanol when stored in room temperature for 24 h, as their UV absorption spectra were found identical to the fresh samples. The standard curve of the drug was prepared in the following media: 1. Distilled water 2. Phosphate buffer of pH 6.8 3. 0.1 N HCL 4. Methanol

Preparation of calibration curve of metformin hydrochloride in different media at 236 nm

Accurately weighed quantity of metformin hydrochloride (10 mg) was dissolved in a small amount of media, and the volume was made up to 100 mL in a volumetric flask. This gave a concentration of 100 μg/mL. Dilutions were prepared as 5, 10, 15, 20, 25, and 30 μg/mL. The absorbance of these was determined at 236 nm by UV spectrophotometric method; values at 236 nm corresponding to each concentration were then statistically evaluated. Calibration curve was plotted taking absorbance on y-axis and concentration on x-axis.

Compatibility study of the drug with different excipients

Each excipient was weighed accurately to 100 mg. Further, 100 mg of metformin hydrochloride was added to them separately. Six sets of the prepared physical mixture were placed in a glass vial, which were hermetically sealed. These vials were further kept at 25°C and 55°C for 2 weeks.[11] The sets were kept for 2 weeks and were opened and observed for caking, liquefaction, discoloration, and odor or gas formation.

Drug–excipient interference studies

To eliminate the chances of excipients interfering with the analysis, the preliminary interaction studies were carried out. A 0.1% wt/vol solution of polymers was prepared by dissolving each excipient separately in the phosphate buffer (pH 6.8). To 1 mL of stock of drug, 9 mL of 0.1% wt/vol polymer solution was added, and the solutions were scanned in UV spectrophotometer from 400–200 nm.

Prototype formula development

Design of formula for novel pulsatile drug delivery system

Selection of method: Extrusion and spheronization technique was used for the preparation of pellets. Isopropyl alcohol (IPA) was used as a binder solvent, and polyvinyl pyrrolidone (PVP) K 30 as a binder solution. Dry powder was blended in the planetary mixture for 25 min. The appropriate quantity of IPA and PVP K30 gave suitable wet mass for extrusion, which was determined by trial and error method. Wet masses were extruded in screen extruder (Caleva model 10, Dorset, England) equipped with standard screen 1 mm diameter aperture and rollers. Further, the extrudates were shifted to spheronizer (Caleva model 250, Dorset, England) equipped with a crosshatch plate (250-mm diameter) processed at 1000 rpm. 500 mg of API was accurately weighed and was taken in 10 different batches. The lag time modifier HPC Type H and Eudragit L/S were used for pH-dependent release; PVP K30; microcrystalline cellulose (MCC); magnesium stearate; talc were taken in 10 batches and were mixed thoroughly.

Coating method used: Ethyl cellulose and triethyl citrate were used for coating the pellets. The final pellets were dried in fluid bed dryer with an inlet airflow of 50°C for 20 min. Post drying, the different size pellets were separated by dry sieving methods with a set of standard sieves (710–1100 μm) with square openings.
Method for formulation strategy of different batches:
Water was used as granulating liquid, and the granules formed were subjected to extrusion and spherization. The spherization speed was 1000rpm, and the spherization time was 3min. The granulating liquid was used in a quantity of 60mL per batch, and IPA was taken in sufficient quantity in each batch. It was performed by the trial and error method[12] as shown in Tables 2 and 3.

In vitro dissolution study: In vitro dissolution study was carried out in USP Type II apparatus in 900mL of different media (HCl pH 1.2 for 2h, the simulated intestinal fluid [SIF] pH 6.8 for the remaining 10h) at 50rpm at 37°C. Samples were withdrawn at regular intervals and analyzed by UV spectrophotometer at 236nm. The aliquot samples were withdrawn at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12h, and were replaced with blank medium to maintain the sink condition.

Evaluation of selected optimized formulation batch 5
The optimized formulation was evaluated for following physical characters:

Angle of repose
Angle of repose was measured by using a funnel having 0.5cm diameter orifice. Pellets of 20g were placed in funnel and were allowed to fall from 4cm on a hard level surface. By determining the height and radius of the resulting pellets, the angle of repose was obtained.

Aerated bulk density
Approximately 20g pellets were poured gradually through a funnel into a 50mL graduated cylinder, tapped lightly on a hard surface, and volume was measured. Bulk density was measured using weight and volume of pellets.

Tapped density
The aforementioned graduated cylinder was measured 500 times using tapped density tester, and tapped volume was measured.

Carr index and Hausner ratio
Carr index and Hausner ratio were determined to study the flowability by Equations (4) and (5).

Percentage friability
The friability test of pellets was done on 5g pellets combined with 5g of glass beads (2mm diameter) using Roche Friabilator (Panomax Inc., New Delhi, India). Friabilator was rotated at 25rpm for 4min. The loss of pellet weight with respect to initial weight was then calculated as percent friability.

Particle size distribution
Sieve shaker method was used to determine PSD. Sieve of different apertures including ASTM 16 (1180 µm), # 18 (1000 µm), # 20 (850 µm), and # 25 (710 µm) mesh size were placed on mechanical shaker. The shaker was shaken for a definite time (20min). The particles retained on different sieve were attained and particle size was calculated [Tables 1 and 4-6].

\[ d = \frac{\sum x_i d_i}{100} \]  (6)

where \( x_i \) is the mean of the upper and lower limits of sieve fraction and \( d_i \) is the % of the fraction.[13]

Surface characteristics
Shape and surface nature of optimized pellets were studied by scanning electron microscope (model JSM T200; Joel, Tokyo, Japan), using gold sputter technique. These particles were further subjected to vacuum, dried, and coated with gold palladium and observed microscopically.

Interaction studies of batch 5 with excipients
To ascertain any kind of interaction of metformin hydrochloride with the excipients and polymers used in the optimized formulation, interaction studies were carried out. In this study, the optimized formulation batch 5, the placebo formulation, and API were subjected to Fourier transform infrared (FTIR) spectroscopy and DSC analysis.

Fourier transform infrared spectrum analysis
In this study, KBr tablet technique was used along with IR spectra of drug placebo formulation. The optimized formulation was recorded and compared [Table 7].

Differential scanning calorimetry
DSC of drug, placebo formulation, and optimized formulations were recorded and compared.

| Table 2: Formulation strategy of different batches |
|-----------------------------------------------|
| Batches                                      |
| Talc (mg)                                    |
| Magnesium stearate (mg)                      |
| MCC (mg)                                     |
| PVP K30 (mg)                                 |
| HPC Type H (mg)                              |
| Batch 1                                      |
| Batch 2                                      |
| Batch 3                                      |
| Batch 4                                      |
| Batch 5                                      |
| Batch 6                                      |
| Batch 7                                      |
| Batch 8                                      |
| Batch 9                                      |
| Batch 10                                     |

| Talc (mg) | 0 | 0 | 0 | 0 | 5 | 5 | 5 | 5 | 5 |
|----------|---|---|---|---|---|---|---|---|---|
| Magnesium stearate (mg) | 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| MCC (mg) | 0 | 0 | 0 | 0 | 0 | 10 | 20 | 30 | 40 |
| PVP K30 (mg) | 0 | 0 | 10 | 25 | 25 | 25 | 25 | 25 | 25 |
| HPC Type H (mg) | 130 | 120 | 110 | 100 | 90 | 80 | 70 | 60 | 50 |

Bold values in the table depict the selected batch number 5 values which was studied further in this research work.
Stability studies of batch number 5

Stability studies were carried out to determine the effect of polymer or additives on the stability of the drug. The determination of physical stability under accelerated stability condition of temperature and humidity was carried out. Further, the study of elevated temperature and humidity on important characteristics of optimized capsules was conducted. The stability studies were carried out according to International Council for Harmonisation guidelines. The sufficient quantity of optimized capsules was kept in aluminum pouches. The pouches were placed in humidity chamber having 40°C ± 0.5°C and 75% relative humidity (RH). Samples were withdrawn at 0, 30, 60, and 90 days. They were studied for the following characteristics.\textsuperscript{[14]}

Accelerated stability study as per WHO guidelines and shelf-life determination

The optimized pellet capsules were placed in an inert glass container and stored at 40°C ± 0.5°C, 50°C ± 0.5°C, and 60°C ± 0.5°C for 90 days [Table 5]. The samples were withdrawn at 0, 30, 60, and 90 days, and were analyzed for their drug content by stability-indicating HPLC method using a standard curve. Log of the remaining drug was plotted against time (in days). Slope of each line was obtained, and degradation rate constant was calculated by the formula:

\[
\text{Slope} = -\frac{K}{2.303}
\]

where \(K\) is the degradation rate constant.\textsuperscript{[15]}

Statistical analysis

The data were subjected to two-way analysis of variance (ANOVA) followed by Bonferroni \textit{post hoc} test. The statistical difference was calculated using the software GraphPad Prism (San Diego, CA, USA).

RESULTS

Preformulation studies

\textit{Organoleptic properties}: Drug was found to be crystalline, white in color, bitter to taste, and odorless.

\textit{Melting point}: The value of the melting point was observed to be 225°C (complied with the reported value, i.e., 224°C–226°C).

\textit{DSC}: Thermogram was shown in Figure 1.
Pandey and Selvamurthy: Design and in vitro characterization of biguanide antidiabetic drug

**Table 5: Values of coefficient of correlation and equations of the best fitting line**

| S. no. | Media          | $R^2$   | Equation                                      |
|-------|----------------|---------|-----------------------------------------------|
| 1     | Distilled water | 0.9992  | $y = 0.0193x - 0.0068$                       |
| 2     | PO₄ pH 6.8     | 0.9994  | $y = 0.0198x - 0.0006$                       |
| 3     | HCl pH 1.2     | 0.9997  | $y = 0.0199x + 0.0006$                       |
| 4     | Methanol       | 0.9999  | $y = 0.02x + 0.0002$                        |

**Table 6: Micromeritic data of optimized formulation**

| S. no. | Parameter              | Mean value ± SD |
|--------|------------------------|-----------------|
| 1      | Angle of repose        | 26.64° ± 0.01°  |
| 2      | Aerated bulk density   | 0.625 ± 0.05 g/cc |
| 3      | Tapped density         | 0.7142 ± 0.04 g/cc |
| 4      | Carr’s index           | 12.489 ± 0.02   |
| 5      | Hausner ratio          | 1.143 ± 0.05    |

**Table 7: Results of compatibility study of metformin hydrochloride with excipients used**

| Drug + excipients | 25°C (2 weeks) | 55°C (2 weeks) |
|-------------------|----------------|----------------|
| Metformin HCl     | No change      | No change      |
| Metformin HCl + HPC Type H | No change | Aggregates |
| Metformin HCl + Eudragit L 100 | No change | No change |
| Metformin HCl + ethyl cellulose | No change | Off white |
| Metformin HCl + magnesium stearate | No change | No change |
| Metformin HCl + Talc | No change | Lumps |

**Figure 1: Differential scanning calorimetry thermogram of metformin hydrochloride**

ESI for metformin hydrochloride. Both signal intensity and signal-to-noise ratio obtained in positive ionization mode were much greater than those in negative ionization mode. The precursor ion in the full scan spectra, and the most abundant ions were protonated molecules $(M + H)^+ m/z; 43, 44, 85, 42, 129, 68, 30$ were observed [Figure 3].

HPLC: The retention time was found to be 5.627 for metformin hydrochloride [Figure 4].

**Analytical methodology of API**

**UV method validation:** The values of the coefficient of correlation and equations of the best fitting line are given in Table 5 and Figure 5.

**Criteria for establishing linearity**

As per the USP, the relative standard deviation (RSD) or the percentage coefficient of variance (% CV) in the spectroscopic analysis should be less than 2% and/or the intercept of the line should be less than 2% of the absorbance of the maximum (100%) concentration.

The correlation coefficient values ($R^2$) and equation of the best fitting for all standard curves are given in Table 5.

High and positive values of the coefficient of correlation ($R^2$) in all cases indicate good linearity within the pharmacopoeial limits. The correlation

**Particle size distribution by sieve method**

**pH solubility analysis:** The pH was measured with pH meter, which was found to be 6.6 and that complied with the reported value of 6.68 as per the certificate of analysis provided by Sun Pharma.

**Identification**

**UV spectral analysis:** The stock solutions of the drug in different media were checked for absorbance in the region of 400–200 nm, and UV absorption spectra were obtained. The aforementioned samples of the drug in various media were also stored for 24 h at room temperature in stoppered bottles, and their UV absorption spectra were again determined.

**FTIR spectroscopy:** The IR spectrum of the drug was measured by using KBr pellet method. The principal peaks at wave numbers 1580, 1620, 1063, 1075, 935, 740 cm⁻¹ were observed due to N–H deformation, N–H deformation, C–N stretching, C–N stretching, N–H out of plane bending, and N–H wagging, respectively, as shown in Figure 2.

**Mass spectroscopy:** The mass spectrometer was tuned in both positive and negative ionization modes with

---

**Table 6: Micromeritic data of optimized formulation**

| S. no. | Parameter          | Mean value ± SD |
|--------|-------------------|-----------------|
| 1      | Angle of repose   | 26.64° ± 0.01°  |
| 2      | Aerated bulk density | 0.625 ± 0.05 g/cc |
| 3      | Tapped density    | 0.7142 ± 0.04 g/cc |
| 4      | Carr’s index      | 12.489 ± 0.02   |
| 5      | Hausner ratio     | 1.143 ± 0.05    |

**Table 7: Results of compatibility study of metformin hydrochloride with excipients used**

| Drug + excipients | 25°C (2 weeks) | 55°C (2 weeks) |
|-------------------|----------------|----------------|
| Metformin HCl     | No change      | No change      |
| Metformin HCl + HPC Type H | No change | Aggregates |
| Metformin HCl + Eudragit L 100 | No change | No change |
| Metformin HCl + ethyl cellulose | No change | Off white |
| Metformin HCl + magnesium stearate | No change | No change |
| Metformin HCl + Talc | No change | Lumps |

**Figure 1: Differential scanning calorimetry thermogram of metformin hydrochloride**

ESI for metformin hydrochloride. Both signal intensity and signal-to-noise ratio obtained in positive ionization mode were much greater than those in negative ionization mode. The precursor ion in the full scan spectra, and the most abundant ions were protonated molecules $(M + H)^+ m/z; 43, 44, 85, 42, 129, 68, 30$ were observed [Figure 3].

HPLC: The retention time was found to be 5.627 for metformin hydrochloride [Figure 4].

**Analytical methodology of API**

**UV method validation:** The values of the coefficient of correlation and equations of the best fitting line are given in Table 5 and Figure 5.

**Criteria for establishing linearity**

As per the USP, the relative standard deviation (RSD) or the percentage coefficient of variance (% CV) in the spectroscopic analysis should be less than 2% and/or the intercept of the line should be less than 2% of the absorbance of the maximum (100%) concentration.

The correlation coefficient values ($R^2$) and equation of the best fitting for all standard curves are given in Table 5.

High and positive values of the coefficient of correlation ($R^2$) in all cases indicate good linearity within the pharmacopoeial limits. The correlation

---

**Table 7: Results of compatibility study of metformin hydrochloride with excipients used**

| Drug + excipients | 25°C (2 weeks) | 55°C (2 weeks) |
|-------------------|----------------|----------------|
| Metformin HCl     | No change      | No change      |
| Metformin HCl + HPC Type H | No change | Aggregates |
| Metformin HCl + Eudragit L 100 | No change | No change |
| Metformin HCl + ethyl cellulose | No change | Off white |
| Metformin HCl + magnesium stearate | No change | No change |
| Metformin HCl + Talc | No change | Lumps |
coefficient values were found to be very near to “1.” Hence, linearity was within pharmacopoeial limits.

Compatibility study of the drug with different excipients
Following observation was given in Table 7.

Drug–excipient interference studies
Not even a single polymer showed absorbance at 236 nm, and the results are shown in Figure 5.

Microscopy and %yield of formulations

Evaluation of selected optimized formulation batch 5
Batch 5 was selected and evaluated for the following physical characters and micromeritic data are shown in Table 6.

Percentage friability: It was found to be 0.33%.

Particle size distribution: The particles retained on different sieve were attained and particle size was calculated [Table 8].

Surface characteristics: Scanning electron microscopy (SEM) showed smooth and round surface of pellets as shown in Figure 6A and B.

Interaction studies of batch 5 with excipients

FTIR spectrum analysis: KBr tablet technique was used and IR spectra of the drug, placebo formulation, and optimized formulation were recorded and compared [Figures 7 and 8].

DSC: Differential scanning calorimeter of the drug, placebo formulation, and optimized formulations were recorded and compared [Figure 9].

Stability studies of batch number 5
Accelerated stability study according to WHO guidelines and the shelf-life determination [Tables 9-11].

Statistical analysis
The data were subjected to two-way ANOVA followed by Bonferroni post hoc test for analyzing the statistical difference using the software GraphPad Prism. [6]

Discussion
The DSC curve of pure metformin hydrochloride showed an initially flat profile, followed by a single sharp exothermic peak. It represented the melting of the substance in the range 222°C–237°C ($T_{\text{onset}} = 231.2$, $T_{\text{peak}} = 233.33$, and $\Delta H_{\text{fusion}} = -311.51$ J/g). The aforementioned DSC thermogram showed an exothermic peak with a melting point at 225.029°C, hence was equivalent to the reported value. The exothermic peak showed the crystalline nature of the drug. The drug was found to have good flow property. From the aforementioned analysis, it was inferred that the average particle size of the API was in the range of 85–150 microns.
The drug was found to have good flow property. The flowability of the particles plays an important role in the filling of the die to a constant volume to give the required mass. Particle size, shape, surface area, density, moisture content, and the electrostatic charge affect the flow properties of the powder. Particle size larger than 250 μm usually flows relatively more freely than 100-μm size powders, whereas powder with particle size less than 10 μm resists flow under gravity because of the presence of the extremely cohesive forces.

The angle of repose was found to be more than 30° for API indicating that API is not having good flow property, and the addition of glidant improved flow property.

The Carr compressibility index gives the relation between flowability and compressibility and can be used to choose a suitable method of pellet manufacturing. It showed very poor flowability of API, and glidant was added to rectify this problem.

The FTIR, HPLC, and mass spectrum showed that the procured API matched the certificate of analysis provided by the manufacturer, hence it was authentic. It was found that the drug did not interact with almost all the polymers and excipients. HPC Type H, ethyl cellulose, and talc interacted only at 55°C, whereas no
interaction was observed at 25°C. Hence, the drug was compatible with polymers or excipients used.

None of the polymers showed absorbance at 236 nm. Thus, it was concluded that the excipients did not interfere with the analysis of the drug. Batch 5 was selected as an optimized formulation having smooth and spherical pellets ideal for filling into the hard gelatin capsule with minimum wastage, and the percentage yield was 78.98%, which was maximum of all batches. After studying the dissolution characteristics of the formulation and the lag time shown by the formulations as aforementioned, batch 5 was selected for further studies, as it showed a lag time of 6 h due to the optimum quantity of HPC type H and better reproducibility, showing pH-dependent release in the pH 6.0 due to Eudragit L 100 and in pH 6.8 due to Eudragit S 100, and showing the gradual release of drug from 6 to 8 h and rapid release of drug from 8 to 10 hrs Figures 10 and 11.

SEM showed a smooth rounded surface of pellets and showed intact surface without any perforations, channels, or troughs due to extrusion and spheronization method. Comparison of DSC thermogram and FTIR showed similar principle peaks for drug, optimized pellets, and placebo. No significant shifts of reduction in the intensity of the FTIR bands of metformin hydrochloride were observed. Hence, both showed no interaction between drugs and polymers. In DSC thermograms, the thermal curves of both binary and ternary mixtures obtained by simple blending corresponded to the superimposition of those of the single components, indicating the absence of solid-state interactions.

Figure 6: (A, B) Scanning electron microscopy of optimized pellets of Batch 5

Figure 7: Infrared spectroscopy of optimized pellets
and allowing the assessment of drug–polymers compatibility in all the examined formulations. As a further confirmation of the absence of any incompatibility problem, no variations in the thermal behavior of samples of binary and ternary combinations were observed after their pelletization and subsequent coating. Thus, no definite solid–solid interaction could be concluded for the examination of all the DSC thermograms. DSC ruled out the occurrence of solid-state interaction and complex formation.\[6\]

From the curve of the figure, the degradation rate constant was determined in days, and it was found to be 1.99% of the drug degradation. The data were subjected to two-way ANOVA followed by Bonferroni post hoc test, and in all the cases, \( P < 0.005 \) was considered as significant. On the basis of the dissolution profiles of various pellet formulations, an inverse relation between the amount of lag time modifier polymer present and the released rate of the drug was observed.

**CONCLUSION**

The pulsatile drug delivery of metformin hydrochloride pellets with timed release was developed and evaluated in our laboratory. The pellets were prepared with different concentrations of lag time modifiers, enteric-coated material using extrusion and spheronization method, which were further optimized. Optimization procedure aided in the preparation of pellets of metformin with lag time up to 6 h. The *in vitro* dissolution studies revealed that the formulated spherical pellets released the desired concentration of the drug at predetermined time points. The optimized formulation was found to be stable, with predicted shelf life of 1.94 years.

**Acknowledgement**

We appreciate the support from Sun Pharma, Gurugram, India, for providing metformin hydrochloride as a gift sample, and Jubilant Generics for providing excipients.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.
Table 9: Effect of storage at 40°C and 75% RH

| S. no | Time (days) | Weight gain (%wt/wt) (n = 10) (±SD) | Assay (%), n = 20 | Mean lag time (h) (±SD) |
|-------|-------------|-----------------------------------|------------------|------------------------|
| 1     | 0           | 0.0                               | 99.97            | 5.50 ± 0.20            |
| 2     | 30          | 0.33 ± 0.21                       | 99.11            | 3.55 ± 0.20            |
| 3     | 60          | 0.67 ± 0.46                       | 98.83            | 4.25 ± 0.30            |
| 4     | 90          | 1.22 ± 0.54                       | 98.14            | 5.25 ± 0.50            |

Table 10: Degradation of metformin hydrochloride in formulation batch 5 at 40 ± 0.5°C and 75% RH

| Time (days) | Mean area value | Concentration (μg/mL) | % of drug remaining in dosage form | Log % of drug remaining in dosage form |
|-------------|-----------------|-----------------------|-----------------------------------|--------------------------------------|
| 0           | 5023            | 49.56                 | 100                               | 2                                    |
| 30          | 5002            | 49.22                 | 99.6                              | 1.99825                              |
| 60          | 4996            | 48.99                 | 99.10                             | 1.99607                              |
| 90          | 4878            | 48.51                 | 98.97                             | 1.99550                              |

Table 11: Degradation of metformin hydrochloride according to WHO guidelines

| Time in days | 40°C ± 0.5°C | 50°C ± 0.5°C | 60°C ± 0.5°C | 1/T days⁻¹ |
|--------------|--------------|--------------|--------------|------------|
| % drug remaining | Log% drug remaining | % drug remaining | Log% drug remaining | % drug remaining | Log% drug remaining |
| 0            | 100          | 2            | 100          | 2          | 100          | 2          | -           |
| 15           | 99.8         | 1.999130     | 99.6         | 1.99825    | 99.5         | 1.99738    | 0.066       |
| 30           | 99.6         | 1.99825      | 99.3         | 1.99695    | 99.1         | 1.99607    | 0.033       |
| 45           | 99.30        | 1.99694      | 99.0         | 1.99565    | 98.8         | 1.99475    | 0.022       |
| 60           | 99.10        | 1.99607      | 98.7         | 1.99431    | 98.4         | 1.99299    | 0.016       |
| 75           | 98.98        | 1.99555      | 98.21        | 1.99216    | 98.02        | 1.99131    | 0.013       |
| 90           | 98.54        | 1.99361      | 97.97        | 1.99109    | 97.45        | 1.98878    | 0.011       |

Figure 10: Dissolution profile for formulation batch 5 in HCl pH 1.2 for 2 h and phosphate buffer pH 6.8 for the remaining 10 h

Figure 11: Dissolution profile for 10 formulation batches in HCl pH 1.2 for 2 h and phosphate buffer pH 6.8 for the remaining 10 h
REFERENCES

1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018;138:271-81.

2. Saigal N. Chronotherapeutics drug delivery system for treatment of hypertension and allergic rhinitis. New Delhi, India: Jamia Hamdard; 2010.

3. Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, et al. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 2011;50:81-98.

4. Pentikäinen PJ. Bioavailability of metformin. Comparison of solution, rapidly dissolving tablet, and three sustained release products. Int J Clin Pharmacol Ther Toxicol 1986;24:213-20.

5. Food and Drug Administration. Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System. . Silver Spring, MD: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); 2000. Available from: https://sci-hub.tw/10.1201/9780824741969.axh. [Last accessed on 2020 July 16].

6. Wadher KJ, Kakde RB, Umekar MJ. Study on sustained-release metformin hydrochloride from matrix tablet: influence of hydrophilic polymers and in vitro evaluation. Int J Pharm Invest 2011;1:157-63.

7. Saeed RN, Amer M, Malik A, Nadeem M, Hassali MA, Irshad N, et al. Formulation development of metformin tablet and its comparative in vitro study with different brands in Pakistan. Int J Pharm Sci Rev Re 2013;19:12-7.

8. Antequera MV, Ruiz AM, Perales MC, Munoz N, Ballesteros MR. Evaluation of an adequate method of estimating flowability according to powder characteristics. Int J Pharm 1994;132:155-61.

9. Chen L, Zhou Z, Shen M, Ma A. Simultaneous determination and pharmacokinetic study of metformin and rosiglitazone in human plasma by HPLC-ESI-MS. J Chromatogr Sci 2011;49:94-100.

10. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Rapid determination of metformin in human plasma using ion-pair HPLC. J Pharm Biomed Anal 2003;31:197-200.

11. Banker GS, Rhodes CT. Modern pharmaceutics. 4th ed. New York: Marcel Dekker; 2002. Available from: https://books.google.co.in/books/about/Modern_Pharma.html?id=s1-BerNQAtsC&redir_esc=y. [Last accessed on 2020 July 16].

12. Quereshi JM. Design and characterization of chronomodulated drug delivery system. New, India: Jamia Hamdard; 2008.

13. Gosar A, Folane S, Pawar S, Gharat M, Lalge A, Jhadav S. Development and validation of new analytical method for the determination of particle size distribution of metformin hydrochloride using laser based particle size analyzer. J Pharm Res Int 2017;17:1-9.

14. Mudasir M, Jan R. Stability studies for the determination of shelf life of aceclofenac formulation. Der Pharm Lett 2012;4:483-6.

15. Banerjee S, Chattopadhyay P, Ghosh A, Bhattacharya SS, Kundu A, Veer V. Accelerated stability testing of a transdermal patch composed of eserine and pralidoxime chloride for prophylaxis against (±)-anatoxin A poisoning. J Food Drug Analysis 2014;22:264-70.