Development and characterization of multiparticulate system as an alternative to unit dosage forms containing drugs with diverse release profiles

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Abstract

Background: Currently, the Bilayer tablets, Tablet in tablet and Inlay tablets composed of Glimepiride (GMP) and Pioglitazone (PGH) as immediate release part, and Metformin (MFH) as sustained-release part are available for the treatment of type II diabetes mellitus (T2DM). In these products, there is a possibility of the incomplete release of immediate release part (GMP and PGH) due to their entrapment into high viscosity gel barrier of MFH sustained-release part when drug product comes in contact with media. Therefore, the present study was aimed to deliver the above combination drugs in the form of hard gelatin capsules (as unit dosage form) containing MFH sustained-release (MFH-SR) pellets and immediate release pellets of PGH and GMP (PG-IR).

Results: The MFH-SR and GP-IR pellets, prepared by extrusion and spheronization technique, were optimized based on the drug content and % cumulative drug release. The MFH-SR pellets formulation (batch A6) has shown maximum drug content, and sustained-release of MFH similar to marketed glucophage tablet while, the GP-IR pellets formulation (batch B5) has displayed maximum drug contents and immediate release of GMP and PGH; thus, these batches were considered for further characterizations. The optimized MFH-SR and GP-IR formulations have shown particle sizes of 0.23 ± 0.0010 mm and 0.35 ± 0.0018 mm, respectively. Besides, the formulations exhibited good micromeritics properties. The in vivo pharmacokinetic study in rabbits has demonstrated comparable bioavailability of the drugs from pellets and the marketed formulations following oral administration. Further, the pellets were found to be stable for 6 months at 40 ± 2 °C/75% ± 5%RH.

Conclusions: The study results revealed that the multiparticulate systems with varied release profiles could be a promising approach to overcome drug release issues associated with the unit dosage forms.

Keywords: Metformin hydrochloride, Glimepiride, Pioglitazone hydrochloride, Pellets, Design of experiment, Pharmacokinetics

Background

The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [1]. Type 2 diabetes mellitus (T2DM) is a multifactorial disease characterized by insulin resistance. Insulin resistance in type 2 diabetes patients has increased the demand for insulin that could not be met by the pancreatic β cells due to defects in their function. On the contrary, insulin secretion decreases with the increased demand due to the gradual destruction of β cells; that could transform type 2 diabetes (T2D) patients from being independent to become dependent on insulin. Most T2D patients are not dependent on...
insulin where insulin secretion continues, and insulin depletion rarely occurs.

The T2DM treatment with monotherapy often does not provide effective glycaemic control, generating the need for add-on therapy. Hence, multiple oral hypoglycaemic agents formulated as a single dosage form called fixed-dose combinations (FDCs) play an essential role in glycaemic control [2]. For these patients, sulphonylureas (glimepiride, GMP), the biguanide (metformin hydrochloride, MFH), and thiazolidinediones (pioglitazone hydrochloride, PGH) are the most prescribed oral treatment options [3]. The sulphonylureas reduce hyperglycemia by enhancing insulin secretion [4] whereas; MFH improves insulin sensitivity and suppresses hepatic glucose output [5]. Because of their complementary mechanism of action, the combination therapy with sulphonylurea, biguanide, and PGH would be rational and may lead to benefits in terms of improved glycemic control and improved tolerability at lower doses of the individual agents [6].

Gastrointestinal absorption of MFH is incomplete with an absolute bioavailability of only 40–60%. In addition, the drug shows rapid elimination with 20–30% of the oral dose recovered in faeces. Studies indicate that administration of a fixed combination of GMP, MFH and PGH was safe and more effective than the individual drug [7]. In addition to a reduction of the pill burden, fixed-dose combination (FDC) has been shown to reduce the dosing frequency and thereby improve adherence. FDC may also neutralize the potential side effects. The potential of weight gain with PGH and sulphonylureas may be neutralized by the weight loss properties of MFH [7–9].

All marketed combination therapies with GMP (immediate release part), PGH (immediate release part) and MFH (sustained-release part) are available in the form of Bilayer tablets, Tablet in tablet and Inlay tablets. In the preliminary investigation, marketed bilayer formulations were evaluated for in vitro dissolution; wherein the incomplete release was observed for GMP and PGH (please refer to Additional file 1). This might be due to entrapment of GMP and PGH into the high viscosity gel barrier of MFH extended-release portion when drug product comes in contact with media. Although such studies are not found in the literature (to the best of our knowledge), we hypothesize that there is a possibility that the extended-release layer of MFH may retard the GMP and PGH release; that may further affect the desired pharmacokinetic parameters and efficacy of unit dosage formulations. Therefore, the present study was aimed to deliver the above combination drugs in the form of hard gelatin capsules (as unit dosage form) containing MFH sustained-release (MFH-SR) pellets, and immediate release pellets containing both PGH and GMP (PG-IR).

Further, pellets can distribute in the gastrointestinal tract homogeneously thus maximize drug absorption and reduce peak plasma fluctuations, minimize the risk of local GI tract irritation and dose dumping, decrease dosing frequency and thus increased patient compliance, improve safety and efficacy of the active ingredient, and offer the possibility of combining several active components, incompatible drugs, or drugs with different release profiles in the same dosage unit [10]. Therefore, the present study was aimed to develop MFH-SR and PG-IR pellets as an alternative to existing unit dosage forms for the effective treatment of T2DM. Further, the developed pellets were optimized based on in vitro characterization, and the optimized pellets were subjected for in vivo pharmacokinetic study in comparison with the marketed formulations.

**Methods**

**Materials**

Metformin hydrochloride (MFH) was gifted by Wanbury Laboratories, India. Glimepiride (GMP) and Pioglitazone hydrochloride (PGH) were gifted by Sun Pharma Ltd, India and Aarti drugs, India, respectively. Carbopol 971P was procured from Noveon, Inc. Germany. Tween 80 was purchased from Croda Pvt. Ltd., India. Isopropyl alcohol was procured from Lee Chang Yung Chemical Corporation, Taiwan. Lactose monohydrate was purchased from DMV International Inc. Netherlands. Sodium starch glycolate and microcrystalline cellulose (Avicel PH 101) were obtained from DMV Fonterra, Germany and FMC Biopolymer Thailand, respectively.

**Preparation and optimization of MFH-SR pellets**

The different solvents and solvents mixture such as water alone, water and isopropyl alcohol (IPA) combination in different ratios (15:85, 20:80, 25:75 and 30:70) were tried for preparing MFH-SR pellets. The granulating system forming satisfactory pellets was optimized and used further. Total nine runs of MFH-SR pellets of different formulation compositions were prepared by using extruder-spheronizer (ACM Process Ltd., E-140 and S-320) (Table 1). Briefly, MFH, microcrystalline cellulose (MCC) and Carbopol 971P were passed through #40 sieve and then mixed in a rapid mixer granulator for 10 min at a slow speed using impellers and keeping the chopper off with granulating liquid phase (purified water: IPA at 25:75 ratio). Extrusion mixtures (dough mass) were formulated to produce a cohesive plastic mass with inherent fluidity permitting flow-through during extrusion. The dough mass was then passed through the extruder with dies of 0.2-mm-diameter and extrusion speed of
10–15 rpm to form extrudates. The extrudates formed were then rolled into pellets in the spheroniser, and the pellets were dried at 50–60 °C for 1 h. Finally, the dried pellets were passed through a suitable mesh (50#) to obtain uniform pellets, and fines were separated from the pellets. The formulation of MFH-SR pellets was optimized by using 3² optimal response surface designs (ORSD). The effect of Carbopol 971P and MCC concentration on the dependent variables such as drug content and in vitro drug release was determined (Table 2).

Table 1 Composition of different formulations of MFH pellets

| Ingredients                          | A1   | A2   | A3   | A4   | A5   | A6   | A7   | A8   | A9   |
|--------------------------------------|------|------|------|------|------|------|------|------|------|
| Metformin Hydrochloride (mg)         | 500  | 500  | 500  | 500  | 500  | 500  | 500  | 500  | 500  |
| Carbopol 971P (mg)                   | 250  | 125  | 125  | 125  | 250  | 375  | 250  | 375  | 375  |
| Sodium citrate (mg)                  | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Citric acid (mg)                     | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Microcrystalline cellulose (Avicel PH 101) (mg) | 342.25 | 248.5 | 342.25 | 436.0 | 248.5 | 436.0 | 436.0 | 248.5 | 373.5 |
| Isopropyl alcohol (mL)               | 54.375 | 52.50 | 54.375 | 56.25 | 52.50 | 56.25 | 56.25 | 52.50 | 54.375 |
| Purified water (mL)                  | 18.125 | 17.50 | 18.125 | 18.75 | 17.50 | 18.75 | 18.75 | 17.50 | 18.125 |

Table 2 Optimization of MFH pellets

| Batch Code | % Drug content (Y1) | %CDR (Y2) after 12 h |
|------------|---------------------|---------------------|
| A1         | 96.23 ± 1.26        | 97.33 ± 2.31        |
| A2         | 94.52 ± 1.97        | 92.11 ± 3.15        |
| A3         | 96.16 ± 2.56        | 97.07 ± 1.56        |
| A4         | 95.56 ± 2.61        | 94.12 ± 1.45        |
| A5         | 96.10 ± 3.33        | 96.20 ± 2.06        |
| A6         | 97.68 ± 2.11        | 97.82 ± 1.87        |
| A7         | 95.54 ± 1.60        | 93.01 ± 2.09        |
| A8         | 96.78 ± 1.87        | 84.66 ± 1.98        |
| A9         | 98.31 ± 1.33        | 90.03 ± 2.40        |

Values are mean ± SD, n = 3

10–15 rpm to form extrudates. The extrudates formed were then rolled into pellets in the spheroniser, and the pellets were dried at 50–60 °C for 1 h. Finally, the dried pellets were passed through a suitable mesh (50#) to obtain uniform pellets, and fines were separated from the pellets. The formulation of MFH-SR pellets was optimized by using 3² optimal response surface designs (ORSD). The effect of Carbopol 971P and MCC concentration on the dependent variables such as drug content and in vitro drug release was determined (Table 2).

Table 3 Composition of different formulations of Glimepiride and Pioglitazone pellets

| Ingredients                                      | Formulation Batches |
|-------------------------------------------------|---------------------|
|                                                 | B1     | B2     | B3     | B4     | B5     | B6     | B7     | B8     | B9     |
| Glimepiride (mg)                                | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    | 1.0    |
| Pioglitazone (mg)                               | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   |
| Lactose monohydrate (Pharmatose 200 M) (mg)     | 80.0   | 80.0   | 92.5   | 92.5   | 63.5   | 96.5   | 96.5   | 63.5   | 92.5   |
| Microcrystalline cellulose (mg)                 | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   | 15.0   |
| Sodium starch glycolate (Primogel) (mg)         | 2.5    | 2.5    | 2.5    | 1.0    | 5.0    | 1.0    | 5.0    | 1.0    | 5.0    |
| Tween 80 (mg)                                   | 0.5    | 0.5    | 0.5    | 0.5    | 0.5    | 0.5    | 0.5    | 0.5    | 0.5    |
| Purified water (mL)                             | 3.0    | 3.0    | 3.5    | 3.5    | 2.8    | 3.6    | 3.6    | 2.8    | 3.5    |

Preparation and optimization of immediate release pellets containing both PGH and GMP (PG-IR pellets)
The water with different concentrations of tween 80 was tried as a granulating system for the preparation of pellets. The tween 80 is used as a hydrophilic non-ionic surfactant and acts as a solubilizer. Thus, 0.5% tween 80 in water is used to solubilize GMP and to achieve its uniform distribution. The granulating system forming satisfactory pellets was considered optimum and used further. Total nine batches of PG-IR pellets of different formulation compositions were prepared by extrusion-spheronization technique (Table 3). Briefly, PGH, lactose monohydrate (Pharmatose 200 M), sodium starch glycolate and MCC were passed through #40 sieve and then mixed in a rapid mixer granulator (RMG) for 10 min at a slow speed using impellers and keeping the chopper off. The weighed amount of GMP was dissolved in the specific quantity of purified water containing tween 80 (0.5%v/v). Further, this drug solution was used to prepare the dough-mass [11]. The extrusion mixtures (dough mass) were formulated to produce a cohesive plastic mass with inherent fluidity permitting flow-through during extrusion. The dough-mass was then passed through the extruder with dies of 0.3-mm-diameter and extrusion speed of 10–15 rpm to form extrudates. The extrudates formed
were then rolled into pellets in the spheroniser, and the pellets were dried at 50–60 °C for 1 h. Finally, the dried pellets were passed through a suitable mesh (40#) to obtain uniform pellets, and fines were separated from the pellets.

The formulation of the pellet was optimized by using 3² ORSD. The effect of lactose monohydrate and sodium starch glycolate concentrations on the dependent variables such as drug content and in vitro drug release were determined (Table 4). The factors, responses and levels used for the preparation MFH-SR and PG-IR pellets are depicted in Table 5.

### Capsule filling

An accurate quantity of optimized MFH-SR pellets equivalent to 500 mg of MFH and PG-IR pellets were weighed and filled manually into hard gelatin capsules "00".

### Characterization of formulated MFH-SR and PG-IR pellets

#### Yield of pellets

The obtained pellets were passed through a suitable sieve to separate oversized (doublets), undersized (fine) pellets, and the weight of the desired pellets was noted. The percentage yield of spherical pellets was calculated with respect to the total weight of the pellets taken for sieving.

MFH pellets were manufactured by extrusion and spheronization technique using a roller diameter of 0.2 mm which produced the uniform pellets of defined size and the fines generated during spheronization were removed using #50. The pellets retained on #50 were considered as good pellets for further processing. On the other hand, GMP and PGH pellets were manufactured by extrusion and spheronization technique using roller diameter of 0.3 mm which produced the uniform pellets of defined size, and the fines generated during spheronization were removed using #40. The pellets retained on #40 were considered as good pellets for further processing. Hence, only over-size pellets of specific sieve #50 for MFH and #40 for GMP and PGH were considered to specify the size of pellets. For MFH-SR pellets, sieve #30 was used to remove oversize pellets and sieve #50 to remove fines. The pellets were passed through sieve #30 passed pellets and sieve #50 retained good pellets which are considered to calculate yield. The pellets were passed through sieve #20 and sieve #40 to remove fines. The pellets were passed through sieve #20 and sieve #40 retained good pellets which are considered to calculate yield.

#### Drug content

Accurately weighed 100 mg pellets of each of the 09 batches were finely powdered using mortar-pestle separately, and the powder was dissolved in methanol using a bath sonication. The solution was then filtered and analyzed using a validated HPLC method with PDA detector (Additional file 1).

#### In-vitro release study

The in vitro drug release profiles from MFH-SR and PG-IR pellets were determined using a USP type I dissolution apparatus using dissolution medium (0.1 N HCL) maintained at 37 ± 0.5 °C and stirred at 100 rpm and was compared with marketed Glucophage tablet (MFH 500 mg), Amaryl tablet (GMP 1 mg) and Actos tablet (PGH 15 mg). The capsules filled with pellets were placed

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### Table 4 Optimization of GMP and PGH pellets

| Batch Code | % Drug content | % CDR after 60 min |
|------------|----------------|-------------------|
|            | GMP | PGH | GMP | PGH |
| B1         | 95.05 ± 2.11 | 93.42 ± 1.85 | 94.19 ± 3.64 | 95.28 ± 4.18 |
| B2         | 95.22 ± 1.22 | 93.16 ± 2.31 | 94.05 ± 2.16 | 95.90 ± 3.25 |
| B3         | 95.22 ± 2.05 | 93.01 ± 1.96 | 95.01 ± 2.35 | 94.81 ± 2.19 |
| B4         | 94.30 ± 2.10 | 94.98 ± 2.61 | 93.69 ± 2.45 | 95.06 ± 2.05 |
| B5         | 96.02 ± 2.60 | 95.23 ± 1.79 | 99.87 ± 1.96 | 98.13 ± 2.76 |
| B6         | 94.25 ± 1.78 | 94.15 ± 1.50 | 95.11 ± 2.20 | 95.11 ± 2.20 |
| B7         | 93.01 ± 1.21 | 94.90 ± 2.02 | 98.82 ± 1.14 | 97.51 ± 2.23 |
| B8         | 92.12 ± 2.37 | 91.07 ± 2.08 | 93.12 ± 2.10 | 92.92 ± 3.81 |
| B9         | 94.09 ± 1.77 | 95.93 ± 2.54 | 97.82 ± 2.47 | 94.05 ± 2.91 |

Values are mean ± SD, n = 3

| Table 5 Variables in 3² optimal response surface designs for pellets

| Independent variables | Levels |
|-----------------------|--------|
|                       | Low | Medium | High |
| MFH pellets           |     |        |
| $X_1$ = Carbopol 971 (mg) | 125 | 250 | 375 |
| $X_2$ = Avicel PH 101 (mg) | 248.5 | 342.25 | 436.0 |
| GMP and PGH pellets   |     |        |
| $X_1$ = Pharmatose 200 M | 63.5 | 80.0 | 92.5 |
| $X_2$ = Primogel      | 1.0 | 2.5 | 5.0 |
| Dependent variables (Response) | | |
| $Y_1$ = Drug content  |        |        |
| $Y_2$ = In vitro drug release | | |
in the basket, and a study was performed in 900 mL of media (0.1 N HCL) maintained at 37±0.5 °C and stirred at 100 rpm. 0.1 N HCl was used as a release medium for in vitro release study as it is considered a more relevant medium for correlation of obtained results with the in vivo study performed under fasting conditions [12]. The 5 mL of samples were withdrawn at regular intervals (0, 1, 2, 4, 6, 8 and 12 h) for MFH-SR pellets, and at 0, 5, 10, 15, 30, 45 and 60 min for PG-IR pellets. An equal volume of fresh medium was added to the dissolution apparatus. Finally, the solutions were filtered using a 0.45-μm millipore filter and analysed using HPLC (Shimpack ODS C18 250 × 4.6 mm, 5 μm; Mobile phase: Acetonitrile: 0.02 M phosphate buffer pH of 6.3 adjusted with orthophosphoric acid (OPA); Flow rate: 1.0 mL/min; Injection volume: 20μL; Wavelength of 254 nm). This HPLC method is validated (refer to Additional file 1).

The similarity and difference factor is calculated using the following equations,

\[ f_2 = 50 \times \log \left( \left[ 1 + \frac{1}{n} \sum (R_t - T_t)^2 \right]^{-0.5} \times 100 \right) \]

\[ f_1 = \frac{\left( \sum |R_t - T_t| / \sum R_t \right) \times 100}{\text{number of time points}} \]

where \( n = \text{number of time points}, R_t = \% \text{ Active Pharmaceutical Ingredient (API)} \text{ dissolved of reference product at time point } t, T_t = \% \text{ API dissolved of test product at time point } t \).

Size distribution analysis
The size distributions of optimized MFH-SR and PG-IR pellets were determined by performing sieve analysis. Briefly, British standard sieves 10, 16, 22, 44 and 60 were taken and arranged in order such that the sieve number 10 (coarser sieve) was on the top, and the sieve number 60 (fine sieve) was at the bottom. Accurately weighed 100 gm of pellets were placed on the stack of sieves, and sieves were shaken for 10 min using a mechanical sieve shaker. Finally, the pellets retained on each sieve were collected separately, weighed, and the mean particle size of pellets was calculated.

Flow properties
The flow properties of optimized batches of MFH-SR and PG-IR pellets were investigated by measuring the angle of repose, bulk density, tapped density and Hausner’s ratio in triplicate by standard official methods [13].

Pharmacokinetic study in rabbits
The animal caring and handling and protocols were approved by the animal ethical committee (12/AB/2018). 12 white rabbits (NZW; 06 male and 06 female) weighing 1.5 to 2.0 kg were used for the study. The rabbits were fasted overnight (12 h) before administration of the formulations but had free access to water. The animals were randomly divided into two groups (A and B) with six animals in each group. Rabbits of group A were administered orally with optimized MFH-SR and PG-IR pellets equivalent to dose calculated on a body weight basis. Further, group B received a marketed Glucophage tablet (MFH 500 mg), Amaryl tablet (GMP 1 mg) and Actos tablet (PGH 15 mg) equivalent to dose calculated on a body weight basis [14]. The pellets and tablets were cut with the tablet cutter, and the weight equivalent to the required dose was administered. Briefly, for administration, the product was placed in the smoothly cut (opened) end of a 3 mL syringe (plastic) pushed it ahead with a plunger toward the base of the rabbits tongue for ingestion, followed by a few draughts (nearly 10 mL) of water. A dose of 0.2 mg/kg of GMP, 100 mg/kg of MFH and 3 mg/kg of PGH were administered to each rabbit. Blood samples (0.5 mL) were retrieved by marginal ear vein puncture at different time intervals (0-predose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16 and 24 h post-dosing) for pharmacokinetic analysis. Blood samples were collected in centrifuge tubes containing sodium citrate (3.4% w/v) as an anticoagulant. To separate the plasma, samples were centrifuged for 10 min at 3500 rpm at room temperature, and plasma was collected [15]. Finally, MFH, PGH and GMP were estimated by the liquid chromatography system consisted of solvent delivery (LC10ADVP), controller (LC10ADVP) and column oven (CTO10ASVP) from Shimadzu (Kyoto, Japan). SIL HTC autosampler from Shimadzu (Kyoto, Japan) was used to inject 20μL aliquots of the processed samples on a Peerless basic C18 (33 × 4.6 mm, 5μ particle size) column kept at room temperature. The isocratic mobile phase, a mixture of methanol:water (containing 0.5% formic acid) 8:2 was delivered at 0.6 ml min into the mass spectrometer’s electrospray chamber. Quantification was achieved by MS. All results were presented as mean±SD values [16].

In vitro stability study
The stability study of the optimized batches of pellets was carried out by storing the formulations in high-density polyethylene bottles for a period of 6 months at accelerated stability conditions (40 ± 2 °C/75% ± 5% RH). At predetermined time intervals (30, 90 and 180 days), the pellets were evaluated in terms of physical appearance, drug content and dissolution profile [17].

Statistical analysis
Data are mentioned as the mean ± standard deviation of three independent experiments. The statistical analysis
was performed by using GraphPad Prism software version 5 (GraphPad Software, Inc., La Jolla, CA, USA). The results obtained were analyzed by one-way ANOVA. p < 0.05 was considered statistically significant.

**Results**

**Preparation MFH-SR and PG-IR pellets**

The preliminary trials were conducted on the selection of the right granulating system which would aid the granulation, extrusion and spheronization process. We used water in combination with IPA in the ratio of 25:75 as a granulating system that showed good granulation, extrusion and spheronization of carbopol 971P and MFH mixture. Thus, the water and IPA ratio of 25:75 is considered optimum as a granulating solvent.

In the development of MFH-SR pellets, the carbopol 971 to MFH ratio of 1:0.50 and granulating fluid (water to IPA ratio of 25:75) is found to be feasible to process the pellets [10, 18, 19]. In the case of preparation of PG-IR pellets, the combination of the water and tween 80 was used as a granulating fluid.

**Optimization of MFH-SR pellets**

**Effect of formulation variables on drug content:** Contour plot (Fig. 1A) and 3D surface response plot (Fig. 1B) are used to reveal the effect of carbopol 971 concentration (A) and MCC concentration (B) on the drug content of pellets. Both variables have demonstrated a positive effect on drug content. The augment in A and B caused an augment in the drug content of pellets. Similarly, the interaction effect of both A and B on the drug content is found to be positive (Augment in A and B caused augment in the drug content). The ANOVA analysis of results yielded F-value 13.12 and p-value 0.0298, which denotes the significance of the quadratic model.

The final equation in terms of coded factors for drug content;

\[
\text{Drug content } (Y_1) = +96.59 + 1.13A + 10.0548B + 10.4622AB \\
+ 0.5913A^2 + 10.9473B^2
\]

**Effect of formulation variables on % controlled drug release:** Contour plot (Fig. 2A) and 3D surface response plot (Fig. 2B) were used to assess the effect of carbopol 971 concentration (A) and MCC concentration (B) on the %CDR from pellets. The augment in A and B caused diminish in %MFH release from the pellets. The interaction effect of both A and B on the in vitro release is found to be negative it means that a decrease in the drug release (sustained-release) will be observed as a result of the interaction of A and B. The ANOVA analysis of results yielded F-value 18.88 and p-value 0.0178 that denotes the significance of the quadratic model. Among 09 batches, batch A6 has displayed high drug content (97.68 ± 2.11%), and sustained release profile of MFH (97.82 ± 1.87%) up to 12 h of study which is comparable with marketed glucophage formulation. Therefore, batch A6 is considered as an optimized batch.

The final equation in terms of coded factors for %CDR;

\[
\%\text{CDR } (Y_2) = +97.30 - 1.40A - 2.45B - 3.88AB \\
- 2.48A^2 - 2.68B^2
\]

**Optimization of PG-IR pellets**

**Effect of formulation variables on GMP and PGH content:** Contour plot (Fig. 3A) and 3D surface response plot (Fig. 3B) were used to assess the effect of GMP concentration (A) and PGH concentration (B) on the GMP and PGH content of pellets. Both variables have demonstrated a positive effect on drug content. The augment in A and B caused an augment in the drug content of pellets. Similarly, the interaction effect of both A and B on the drug content is found to be positive (Augment in A and B caused augment in the drug content). The ANOVA analysis of results yielded F-value 13.12 and p-value 0.0298, which denotes the significance of the quadratic model. The final equation in terms of coded factors for drug content;

\[
\text{Drug content } (Y_1) = +96.59 + 1.13A + 10.0548B + 10.4622AB \\
+ 0.5913A^2 + 10.9473B^2
\]
plot (Fig. 3B), and (Fig. 4A) and 3D surface response plot (Fig. 4B) are used to reveal the effect of lactose monohydrate (Pharmatose 200 M) concentration (A) and sodium starch glycolate (Primogel) (B) concentration on the GMP and PGH content of pellets. The augment in A caused a decrease in GMP content while an increase in B caused an increase in GMP content of pellets. The combined effect of A and B on GMP content is found to be negative; it means a decrease in the GMP content. The augment in A and B caused an increase in the PGH content. While the combined effect of A and B on PGH content is found to be negative; it means a decrease in the PGH content. The ANOVA analysis of results yielded F-value 34.87 and p-value 0.0074 that denotes the significance of the quadratic model.

The final equation in terms of coded factors for GMP content;
The ANOVA analysis of results yielded F-value 89.22 and p-value 0.0018 that denotes the significance of the quadratic model. The combined effect of A and B has resulted in decreased PGH content.

The final equation in terms of coded factors for PGH content:

\[
\text{PGH content (Y1)} = +93.50 + 0.7495A + 1.20B \\
- 0.8807AB - 2.13A^2 + 2.51B^2
\]

Effect of formulation variables on % cumulative release of GMP and PGH: The contour plot and 3D surface response plots (Fig. 5A, B) and (Fig. 6A, B) are used to reveal the effect of lactose monohydrate (Pharmatose 200 M) concentration (A) and sodium starch glycolate (Primogel) (B) concentration on the % cumulative release profile of GMP and PGH. Factor A displayed a negative effect on GMP and PGH release; it means increase A will decrease the GMP and PGH release. In contrast, factor B elicited a positive effect; it means an increase in A will increases GMP and PGH release. The combined effect of A and B is found negative; it means an increase in both A and B will decreases GMP and PGH release.

The ANOVA analysis of results yielded F-value 104.32 and p-value 0.0015 that denotes the significance of the quadratic model for % cumulative release of GMP:

\[
\%\text{CDR (Y2)} = +94.63 - 0.1154A + 2.62B - 0.7439AB \\
- 1.86A^2 + 0.1527B^2
\]

The final equation in terms of coded factors for % cumulative release profile of PGH:

\[
\%\text{CDR (Y2)} = +95.97 - 0.3742A + 2.06B - 0.5833AB \\
- 1.94A^2 + 1.87B^2
\]

Both variables (A & B) demonstrated mixed (decreased and increased, respectively) effect on cumulative release of GMP and PGH from IR-pellets. The combined effect of A and B has resulted in decreased release of both GMP and PGH from PG-IR pellets.

Characterization of optimized batches MFH-SR and PG-IR pellets

Yield of pellets
The yield of optimized MFH-SR-A6 pellets and PG-IR-B5 pellets is found to be 93.8 ± 0.55% and 94.6 ± 1.31%, respectively.

Particle size
The particle size of optimized MFH-SR-A6 pellets and PG-IR-B5 pellets is found to be 0.23 ± 0.02 mm and 0.35 ± 0.01 mm, respectively.
Flow properties
The derived properties such as bulk density, tapped density and angle of repose of optimized MFH-SR-A6 pellets are found to be 0.71 ± 0.010 g/mL, 0.81 ± 0.02 g/mL and 27.6 ± 0.76°, respectively. Besides, Carr’s index and Hausner’s ratio are found to be 11.24 ± 0.65% and 1.10 ± 0.11, respectively. On the other hand, the optimized PG-IR-B5 pellets displayed bulk density of 0.83 ± 0.02 g/mL, tapped density of 0.88 ± 0.027 g/mL, angle of repose of 29.2 ± 0.65°, Carr’s index of 12.43 ± 0.73% and Hausner’s ratio 1.24 ± 0.34.

In vitro release study of optimized MFH-SR-A6 and PG-IR-B5 pellets in comparison with marketed products
The in vitro release study of optimized MFH-SR-A6 (Fig. 7A) and PG-IR-B5 (Fig. 7B) pellets filled in a hard
gelatine capsule was studied by using USP type I dissolution apparatus, and results are compared with the marketed tablets. The % cumulative MFH released from MFH-SR pellets and marketed Glucophage tablet is found to be 97 ± 1.8% and 100 ± 3.5%, respectively, after 12 h study. The % cumulative GMP released from PG-IR pellets and marketed Amaryl tablet is found to be 100 ± 4.6% and 97 ± 3.7%, respectively, after 60 min of study. Similarly, the % cumulative PGH released from PG-IR pellets and marketed Actos tablet is found to be 100 ± 4.1% and 99 ± 3.9%, respectively, after 60 min of study.

The difference factor (F1) and similarity factor (F2) are generally used to assess the similarity and bioequivalence among the formulations. For MFH release from optimized MFH-SR (A6) and marketed glipizide formulation, the F1 is observed to be 6 (standard: 0–15), and F2 is found to be 69 (standard: more than 50); indicate similarity and bioequivalence between MFH-SR (A6) test and marketed glipizide reference formulations. Similarly, in the case of the optimized PG-IR (B5) test and marketed amaryl and actos reference formulations the F1 is observed to be 5, and F2 is found to be 70; demonstrate the similarity and bioequivalence between PG-IR (B5) test and marketed amaryl and actos reference formulations.

In vivo pharmacokinetic study

The pharmacokinetic (PK) characteristics of pellets were studied in male white rabbits in comparison with reference formulations. The key PK parameters including $C_{\text{max}}$, $[\text{AUC}_{0-\infty}]$, and $T_{\text{max}}$ are analysed, and the obtained results are presented in Table 6.

In vitro stability study

There is no difference in the physical appearance, drug content and dissolution profile is observed with the optimized pellets after 1, 3 and 6 months of storage at 40±2 °C/75±5%RH. After single time point (60 min) dissolution study (performed after 1, 3 and 6 month storage), %GMP and %PGH release is found to be in the range of 98–100% (about same as before storage). Similarly, there is no much change in the sustained release profile of MFH from the optimized MFH-SR-A6 pellet formulation is observed after 1, 3 and 6 months of storage (refer to Additional file 1).

Discussion

In the present study, the multiparticulate system was developed and characterized as potential approach to overcome the possibility of incomplete drug release from the unit dosage forms with diverse release profiles.

Table 6 Pharmacokinetic parameters of pellet formulations

| Pharmacokinetic parameter | MFH pellets | MFH Reference | GMP pellets | GMP Reference | PGH pellets | PGH Reference |
|---------------------------|-------------|---------------|-------------|---------------|-------------|---------------|
| $C_{\text{max}}$ (ng/mL)  | 1278.8 ± 7.27 | 1280 ± 8.93   | 190.2 ± 3.5 | 195.5 ± 2.9   | 296.8 ± 3.7  | 289.7 ± 2.8   |
| $[\text{AUC}_{0-\infty}]$ (ng.h/mL) | 22,393.53 | 22,769.1   | 1921.25 | 1976.375 | 3528.675 | 3946.125 |
| $T_{\text{max}}$ (h)     | 8.0 ± 0.00   | 8.0 ± 0.00    | 1.5 ± 0.00  | 1.5 ± 0.00    | 2.5 ± 0.00   | 2.5 ± 0.00   |

Values are mean ± SD, n = 3
Initially, the difficulty was observed in the granulation of carbopol 971P and MFH mixture using water alone. This is because the carbopol 971P has resulted in a very tacky mass in presence of water alone. Thus, water alone was found to be unsuitable as a granulating system. The Carbopol 971 was used as a release controlling polymer that sustains the release of MFH from the pellets. The MCC has the ability to absorb and retain a large quantity of water because of its large surface area and high internal porosity; thus, it is used to aid the extrusion spheronization process [10, 16, 18].

Both MFH-SR and PG-IR pellets were optimized by using 3² ORSD. The MFH-SR and GP-IR pellets were optimized on the basis of drug content and % cumulative drug release. The batch A6 of MFH-SR pellets has shown maximum drug content and sustained-release of MFH while, the batch B5 of GP-IR pellets has displayed maximum drug contents and immediate release of GMP and PGH; thus, these batches were considered as optimum.

The flowability of a pellet is of vital significance in the production of pharmaceutical dosage forms to get a uniform feed as well as reproducible filling of capsules to avoid high dose variations. The angle of repose of blend is found in the range of 25°–30° revealing the excellent flowability. In addition, the Carr’s index and Hausner’s ratio values are found within the standard limit indicating good flow property of the prepared pellets [20, 21].

The in vitro dissolution of MFH from MFH extended release tablets (developed using carbopol as release controlling polymer) is found to be independent on pH of dissolution medium (0.1 N HCl, pH 4.5 Acetate buffer and pH 6.8 phosphate buffer) [12]. Thus, in the present study the in vitro dissolution is performed in 0.1 N HCl. On the basis of above information and with the intent to perform fasted bioequivalence study between reference and test MFH-ER pellets to demonstrate in vivo comparison, in current the research work 0.1 N HCl was considered as biorelevant medium and used as dissolution medium. The MFH-SR pellets have demonstrated a sustained release profile due to the presence of release rate controlling polymer Carbopol 971P. The GMP and PGH release profiles from PG-IR pellets are found similar to marketed tablets. Further, the incorporation of excipients (sodium starch glycolate) in the formulation might facilitate the rapid wetting and disintegration of pellets resulting in faster dissolution.

MFH-SR pellets have high dose of 500 mg, hence extruder die of 0.2 mm was used to accommodate larger fill weight of MFH-SR pellets. For Glimepiride and Pioglitazone HCL, IR pellets dose is 1 mg and 15 mg, respectively, where 0.3-mm-die was selected to accommodate 100 mg IR pellets.

In the in vivo pharmacokinetic study, the $C_{\text{max}}$, $AUC_{0-\infty}$ and $T_{\text{max}}$ values calculated from the plasma concentration–time profile for MFH-SR and PG-IR pellets are found comparable with marketed formulations. The stability study results revealed the good stability of pellets under accelerated conditions. Overall, the current study results indicate that the hard gelatin capsules containing MFH-SR and PG-IR pellets could be developed as a potential alternative to tablets containing single or multiple drugs with different release profiles.

Conclusions
At present, there is no evidence in the medical literature (to the best of our knowledge) for MFH, PGH and GMP combinations in the form of multiparticulate delivery systems. Thus, in the present research, MFH-SR and PG-IR pellets were successfully developed and characterized. The micromeritic characteristics of developed multiparticulate systems are found suitable for the industrial development of hard gelatin capsules filled with these multiparticulate systems with different release profiles. Further, the in vitro release and pharmacokinetic profiles of MFH, PGH and GMP obtained from the developed multiparticulate systems are found comparable with the marketed tablet formulations. Therefore, the hard gelatin capsules containing multiparticulate systems with different release profiles could be developed industrially as an alternative to tablets containing an individual drug or multiple drugs with different release profiles; further, this approach improves the patient convenience and compliances and helps to tackle the drug release and bioavailability issues associated with bilayer tablets, tablet in tablet and inlay tablets of MFH, PGH and GMP fixed-dose combinations.

Abbreviations
ANOVA: Analysis of variance; CDR: Cumulative drug release; GMP: Glimepiride; HPLC: High pressure liquid chromatography; IPA: Isopropyl alcohol; MCC: Microcrystalline cellulose; MFH: Metformin hydrochloride; MFH-SR: MFH sustained-release; PGH: Pioglitazone hydrochloride; PG-IR: PGH and GMP immediate release; ORSD: Optimal response surface designs.

Supplementary Information
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Additional file 1. Validation method.

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VD: Performed all above research activities & involved in the preparation and
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Declarations

Ethics approval and consent to participate
All animal procedures and experimental protocol were strictly followed as
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Competing interests
The authors declare that they have no conflict of interests.

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