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ORIGINAL ARTICLE **OPEN ACCESS**

Formulation and Evaluation of Controlled Release Gastro-retentive Floating Bilayer Tablets of Metformin Hydrochloride and Repaglinide for the Treatment of Diabetes

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ABSTRACT
The primary goal of the current research was to formulate controlled release gastro-retentive floating bilayer tablets (GR-FBT) with a controlled release layer of repaglinide (RG) and an immediate release layer of metformin hydrochloride (MH) to maximise the efficacy of both medications when used together for the treatment of diabetes. This study's objective was to evaluate the impact of a polymers on the bilayer tablets. The study also sought to examine the therapeutic effects of combining two medications with immediate and controlled release patterns. A number of in-vitro and in-vivo parameters, including thickness, drug content, weight variation, hardness, friability, disintegration study, swelling index, in vitro floating study, in vitro drug release study, stability study, and in vivo studies, were assessed for the bilayer tablets, which were prepared using direct compression technology. The bilayer tablets had a floating behaviour during the in-vitro dissolution experiment, staying afloat on the test medium for more than 24 hours with a lag time of 4.25 minutes. The in vivo study conducted on Albino rabbits demonstrated favorable absorption of Repaglinide from the bilayer tablets, resulting in improved bioavailability. The pharmacokinetic analysis revealed a significantly lower C_{max} and a significantly higher T_{max} compared to Reference conventional tablets. Additionally, the bilayer tablets exhibited a significantly longer mean residence time and half-life. Remarkably, the in vitro drug release data showed a strong correlation (R=0.901) with the in vivo drug absorption, indicating that the in vitro dissolution test could effectively predict the drug's absorption behavior. This finding highlights the potential of utilizing in vitro-in vivo correlations for predicting drug absorption characteristics.
Keywords: *Controlled release, Floating, Gastro-retentive, Metformin Hydrochloride, Repaglinide*

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INTRODUCTION

Diabetes mellitus [1] is a chronic condition that needs ongoing medical attention. Because of the serious complications it can bring, such as high blood pressure, cardiovascular risk, high cholesterol, kidney disease and retinopathy, diabetes mellitus has risen to the rank of the sixth greatest cause of mortality [2]. In order to control the consequences like lower mortality, and lower health care costs, Type 2 Diabetes prevention and treatment have thus emerged as one of the major problems of the twenty-first century [3]. The amount of glycosylated haemoglobin (HbA1c) and the degree of long-term glycemic control attained are used to measure the efficacy of a diabetic treatment strategy. According to various studies [4-5], the risk of microvascular problems was lowest when HbA1c was in the normal range (4.0–6.0%). A decrease in HbA1c also contributed to this risk reduction.

When compared to their respective conventional dosage forms, controlled drug delivery systems release an active component at rates that are entirely distinct [6]. This System aims to control drug administration, prolong therapeutic activity, and target specific tissues. These systems' drug release has to be predictable, repeatable, and at the desired rate [7].

The definitions of the word "optimize" is "to perfect. The phenomena of optimization is the search for "the best" possible composition [8]. Optimization utilising the Response Surface Method (Central Composite Design) is a robust, effective, and systematic process that shortens the time required for the development of pharmaceutical dosage forms. The use of CCD streamlines the tedious evaluation process for many parameters and their interactions, which enhances research and development efforts by lowering the

number of experimental trials required. The development of pharmaceutical formulations has benefited greatly from the use of CCD, which is thought to be the very effective method for assessing the influence of individual variables with their interactions while requiring the fewest number of experiments [9]. The term "antidiabetic combinations [10]" refers to drugs that contain two or more kinds of antidiabetic medications (each having a unique mechanism of action) in a single tablet or dose. One tablet may be enough to increase compliance and enhance glycemic control.

Drugs benefit greatly from a gastroretentive drug delivery system [11] that has a prolong stomach residence time, including those with an upper GIT absorption window and stomach-acting properties. This method additionally has the ability to speed up administration times by extending drug actions, reduce drug lower-tract inactivation, boost drug impacts on lower intestine flora, and continuously deliver drugs into the small intestinal tract [12]. A floating drug delivery system is seen to be the most promising gastro-retentive dosage form because it doesn't impair the GIT's motility.

The bilayer tablet [13] with two independent release-layers is a formulation that tries to concurrently release two different medications or deliver a drug at two different rates with the following benefits; a formulation of incompatible drugs can be prepared, desirable release pattern can be achieved with simultaneously releasing drugs, reduce dosing frequency and improving patient compliance and adherence.

MATERIAL AND METHODS

The Repaglinide was supplied by Yarrow Chem Products, Mumbai. Metformin Hydrochloride was received as a free sample from Cipla Pharmaceuticals Ltd. Mumbai. Colorcon Co., Ltd. India provided the hydroxypropyl methylcellulose (HPMC K-100M), polyvinylpyrrolidone (PVP K30), crospovidone, and eudragit RSPO, and anhydrous lactose, sodium bicarbonate, microcrystalline cellulose (MCC-101), citric acid, talc, and magnesium stearate were purchased from SD Fine Ltd., Mumbai. All other chemicals used were of analytical quality.

Compatibility studies of drug and polymers [14]

According to the ICH recommendations, the drugs and excipients were mixed in a 1:1 ratio and kept in sealed vials for a period of 4 weeks at a temperature of 40°C and a relative humidity of 75%. By using FTIR methods, the compatibility between the drug-drug and drug-polymer was investigated.

Fourier transform infrared spectrometry (FTIR)

In order to investigate potential drug-drug and drug-excipient interactions, FTIR studies was performed using a Bruker ALPHA II FTIR spectrometer. The FTIR spectra of MH and RG were recorded and analysed to detect any possible interactions between them. Additionally, to assess drug-excipient interactions, the FTIR spectra of pure drugs were obtained in the presence of various polymers. Dry potassium bromide (100 mg) was added to a combination of the drugs and polymers to create potassium bromide pellets for examination. The resulting mixture was then subjected to FTIR spectroscopy. By scanning the samples in the 4000-400 cm^{-1} wavenumber region with a resolution of 4 cm^{-1}, FTIR spectra were produced.

Formulation and optimization of Gastro-retentive Floating bilayer tablets (GR-FBT)

Preparation and Evaluation of Repaglinide solid dispersion (RG-SD)

The RG-SD was prepared using the solvent evaporation technique [15]. This technique involved precise 1:10 weight measurements of RG and PVP K30. The solvent, anhydrous ethanol, was then incorporated into the mixture consisting of RG drug and PVP K30. The solution was heated to 60 °C on a water bath while being vigorously stirred. During the process, the solvent was evaporated due to the heat and stirring, resulting in the formation of a solid product. To remove any residual solvent and further enhance the solid dispersion, the product was subsequently dried out in a vacuum oven. After drying, the RG-SD was crushed to reduce the particle size and improve homogeneity. It was then further dried in a vacuum for a duration of 24 hours. Following this, the solid dispersion underwent pulverization and sieving.

Optimization of RG-CR layer using Central Composite Design (CCD)

The RG-CR layer's optimisation was carried out using Design Expert Software (Version 11). Preliminary trial batches (PTB) of RG-CR layer were prepared for the identification of independent variables that affect the integrity of tablet, total floating time and floating lag time.

Formulation of RG-CR layer

The preparation of the RG-CR layer involved the utilization of direct compression technology [16]. In this process, several components were employed, including 22 mg of RG-SD, matrix forming agent like Eudragit RSPO, HPMC K-100M, and Xanthan Gum, and various other excipients like anhydrous lactose, sodium bicarbonate, microcrystalline cellulose, and citric acid. To begin, each component, including the RG-SD and the matrix forming agent, was separately processed through a mesh with a size of #16. After processing, the components were mixed together for 15 minutes to achieve a homogenous blend. Subsequently, the

lubricants and glidants were also mixed to the blend, and the blend was further agitated for a further five minutes. The resulting blend was then compressed directly into the tablets.

Optimization of MH-IR Layer

The batches of MH-IR layer (MH-1 to MH-9) was prepared and evaluated for various parameter as shown in Table 3.

Formulation of MH-IR Layer

The MH-IR layer was prepared using direct compression technology [16] as shown in Table 2. In this process, MH and microcrystalline cellulose (MCC) were mixed with superdisintegrants for a duration of 15 minutes. The mixture was then passed through a #60 sieve. After sieving, the mixture was then combined for a further five minutes with talc and magnesium stearate. The prepared blend was then processed for direct compression. During the compression process, a constant compression force was applied to ensure consistency across all formulations.

Formulation of GR-FBT

For the formulation of GR-FBT, an optimized batch of MH (MH-2) and an optimized batch of RG were selected as shown in Table 4. The powder blend of the RG-CR layer, consisting of the optimized RG batch, was prepared separately. Similarly, the powder blend of the MH-IR layer (batch MH-2) was also prepared separately. To create the GR-FBT, the die cavity of the rotary tablet machine was initially filled with the powder blend of the RG-CR layer. A single rotation of the machine was performed, compressing the RG-CR layer. Next, the MH-IR layer (batch MH-2) was filled over the compressed RG-CR layer. Both layers were then compressed together to form the GR-FBT. The compression was achieved through direct compression [17].

Characterization of GR-FBT

Thickness

A Digital Vernier calliper was used to test 10 GR-FBT tablets, and the mean and standard deviation readings were reported.

Determination of drug content

RP-HPLC was employed to determine the drug content [18] of MH and RG in the GR-FBT. The mobile phase consisted of a mixture of acetonitrile and potassium dihydrogen phosphate buffer in a ratio of 80:20 (v/v). orthophosphoric acid was used for the adjustment of pH (3.0).

Weight Variation

Total twenty GR-FBT were chosen, and their average weight was determined to evaluate weight variation amongst formulations. The measured weights were compared to the standard range specified in the Indian Pharmacopoeia, which is 5%.

Hardness

To assess the hardness of GR-FBT tablets, a Monsanto hardness tester was used.

Friability

Pre-weighed twenty GR-FBT tablets had performed the Roche friability test, the apparatus was rotating at 25 rpm for 4 min. The tablets being dusted off, and being reweighed. The percentages of weight reduction and friability were recorded.

The percentage friability was determined using the following formula.

$$\mathrm{F} = \frac{\text{Initial Weight of GRFBT} - \text{Final Weight of GRFBT}}{\text{Initial Weight of GRFBT}}$$

Disintegration study

A total of six MH-IR layer were used for the disintegration test using a Disintegration Tester in 0.1N HCl at a temperature of 37±0.5 °C. The time required for the MH-IR layer to completely disintegrate was measured and recorded.

Swelling Index (SI)

The swelling index [19] of RG-CR layer was estimated. The experiment was conducted in triplicate. A pre-weighed RG-CR layer was placed in a beaker having 900 mL of 0.1N HCl solution. Next, a water bath with a regulated temperature of 37±0.5 °C was used to put the beaker. At specified time intervals, the swollen RG-CR layer was carefully taken out from the solution and additional liquid was eliminated by gently dabbing with a filter paper. The RG-CR layer was then weighed again to determine its final weight after swelling.

$$SI = \frac{\text{Final Weight of the RGCR layer} - \text{Initial weight of the RGCR layer}}{\text{Initial weight of the RGCR layer}}$$

***In-vitro* floating study**

The USP type II apparatus (Electrolab India) was used to carry out the *in-vitro* floating study [20] of the GR-FBT. 1000 ml of 0.1N HCl solution were added to the device, which was then kept at a temperature of 37±0.5

°C and a rotational speed of 50 rpm. The total floating time and the floating lag time (FLT) were recorded for each GR-FBT (n= 6).

***In-vitro* drug release study**

In-vitro drug release test [21] was performed for GR-FBT using USP Type II dissolution apparatus (Electrolab, India). Both MH-IR layer and RG-CR layer dissolution studies was performed. Drug samples for MH-IR layer were taken at 0, 0.25, 0.5, 0.75 and 1h and drug samples for RG layer were taken at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 h. The amount of MH and RG was then measured with a RP-HPLC method [18].

Stability study

The GR-FBT were kept in two distinct ways under two different conditions 25 ± 2°C / 60 ± 5% RH and 40 ± 2°C / 75 ± 5% RH for the stability studies22. These conditions were selected to simulate accelerated aging and to monitor any potential changes in the tablets over a period of six months as per the regulation of ICH.

***In-vivo* study**

Animal experiments were carried out in accordance with the approved protocols from the Institutional Animal Ethical Committee (IAEC) of Adina Institute of Pharmaceutical Science, Sagar. The IAEC number assigned to the study is 1546/PO/E/S/11/CPCSEA.

***In-vivo* gastro-retentive study**

To evaluate the gastro-retention property [23] of the GR-FBT, an X-ray imaging investigation was conducted on Albino rabbits (n = 3). In this particular study, a contrast agent (barium sulfate) was substituted for the drug to enhance the visibility of the tablets under X-ray imaging. The other ingredients of the GR-FBT remained consistent, with only the drug being replaced. The rabbits underwent a 12-hour fasting period before the experiment. The GR-FBT was administered to each rabbit using a pet piller, followed by the administration of 30 mL of water. Using an X-ray machine, X-ray photographs were captured at different time intervals during the 2^{nd}, 8^{th}, and 24^{th} hours.

Determination of Pharmacokinetic parameter [24]

Six Albino rabbits (2 and 2.5 kg) were employed for this investigation, the rabbits were allocated into two groups at random. Group A received GR-FBT, while Group B was administered a reference conventional tablet (RCT) with a dose equivalent to the body weight of the animals (MH 11.01 mg/kg and RG 0.09 mg/kg) as shown in Table 7. The tablets were administered to the rabbits using a pet piller. Blood samples of approximately 0.5 ml each were collected from the marginal ear vein at regular intervals. For Group A, the time points for blood sampling were 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, and 36 hours. For Group B, the time points were 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours.

Sample preparation:

Venous blood samples (0.5 ml) were collected from the ear vein into heparinized tubes at specified time intervals. After centrifugation at 4000 rpm for 10 minutes, the resulting supernatant plasma was stored at -70°C. MH and RG were extracted from the serum using ethyl acetate and 0.1N hydrochloric acid as the solvent extraction method [25]. The mixture was vigorously mixed and then 1 mL of a methanol:phosphate buffer solution with pH 4.0 was added. The solution was mixed again and centrifuged at 10,000 rpm for 15 minutes. The resulting supernatant was carefully transferred to a 1.5 mL Eppendorf tube and dried at 65 ºC for 90 minutes. After drying, the sample was reconstituted using mobile phase before analysis. For the RP-HPLC analysis [18], a mobile phase consisting of acetonitrile and phosphate buffer (pH 4.0) in a ratio of 60:40% v/v, with 1% triethylamine, was used. The mobile phase flowed at a rate of 0.8 mL/min, and the drug concentrations were determined at a fixed wavelength of 254 nm. Plasma samples were then analysed using RP-HPLC to determine the drug concentration. Kinetica (PK/PD) analysis 5.0 software was used to analyse the pharmacokinetic parameters, such as C_{max}, T_{max}, $AUC_{0\text{-}t}$, $t_{1/2}$, and MRT.

***In-vitro-In vivo* correlation studies**

In order to establish a mathematical model that relates the in-vitro properties of GR-FBT to the corresponding in-vivo responses, an *in vitro-in vivo correlation*[24] (IVIVC) was employed (Level A). This correlation involved analysing the percentage of drug release in relation to the fraction of in vivo absorption, using a 0.1N HCl medium.

RESULTS AND DISCUSSION

Compatibility studies of drugs and polymers

FTIR investigation is an important analytical technique for assessing the purity and integrity of drugs and for detecting any potential drug-drug interactions that may impact drug efficacy and safety. The figure 1 shows the FTIR spectra of pure Metformin Hydrochloride (MH) and Repaglinide (RG) in 1:1 mixture. According to the observation made, no significant changes were found in the principal peaks of the pure MH and RG drugs. This indicates that the pure drugs maintained their chemical integrity throughout the analysis, and there were no detectable drug-drug interactions between MH and RG. The figure 2 showed

the spectra of pure Metformin Hydrochloride (MH) with mixture of excipients and the figure 3 illustrated the FTIR spectra of pure RG with mixture of excipients. The pure MH and pure RG samples were analyzed by FTIR, and their spectra were compared to the spectra of their respective mixtures with excipients and no significant changes were detected in the FTIR peaks of both drugs with mixture of excipients. It can be concluded that the excipients are compatible with the drugs and do not affect their chemical structure.

Formulation of GR-FBT

Preliminary experimental studies to identify factors and factor levels

Preparation of preliminary trial batches (PTB) was carried out to identify the independent variables that impact the floating lag time, total floating time, and tablet integrity. Based on the observations of the preliminary trial batches (Table 1). The use of Precirol ATO 5 as a polymer in combination with HPMC K-4M, HPMC E-50 LV, and HPMC E-5 LV did not result in a floating tablet. Additionally, when Precirol ATO 5 was used in combination with Carbopol 934, the tablet did not float and also broke. On the other hand, when Eudragit RSPO was used in combination with Carbopol 934, the tablet was able to float, but it dissolved completely within 12 hours. When Eudragit RSPO was used with HPMC K-100M, the tablet was able to float for up to 26 hours. Therefore, based on these observations, Eudragit RSPO and HPMC K-100M in combination could be explored to develop a floating tablet that maintains its integrity for a longer period of time. Eudragit RSPO and HPMC K-100M were finally selected together with other tablet excipients for the manufacture of the RG-CR layer based on the criteria of good tablet integrity, minimal floating lag time, and optimal total floating time.

Formulation and Optimization of RG-CR layer

The experimental design discussed in our prior report[26] was developed using the (3^3) central composite design. Briefly, a centre point was added to create a three-factor (A, B, C), three-level (-1, 0, +1) design. Using CCD, it was determined how independent factors, such as Eudragit RSPO (A), HPMC K-100M (B), and $NaHCO_3$ (C), affected DR1.5, DR8, DR24, and FLT, respectively. Direct compression technique was used to construct the optimised RG-CR layer and to identify the optimal concentrations of Eudragit RSPO, HPMC K-100M, and $NaHCO_3$.

Formulation and Optimization of MH-IR layer

MH-IR layer was optimized by combining Metformin Hydrochloride with the appropriate quantities of crospovidone, croscarmilose sodium and sodium starch glycolate as shown in Table 3, the MH-IR formulations are optimized to ensure rapid drug release. Batch MH-2 containing Crosspovidone (2.5%) disintegrated in 38 sec and releases more than 85% drug in 30 min. and releases 100% drug within 50 min. as shown in Figure 3.

Formulation and Characterization of GR-FBT

An optimized batch of MH-IR (MH-2) and RG-CR (optimized batch) was selected for formulation of the Gastro-retentive (Floating) bilayer tablets through direct compression method. The GR-FBT formulation underwent post-compression evaluation, revealing the results as shown in Table 5. The thickness of the prepared tablet was 3.4±0.05 mm, the hardness was 7.6±0.61 units, and the friability was 0.354±0.002%. The weight variation values all fell within the acceptable limits (5%) set by the Indian Pharmacopeia for it. The drug content analysis indicated the drug content 99.72%±2.41 (MH-IR layer) and 99.16±3.21 (RG-CR layer). The disintegration time for MH-IR layer was found to be 33±1.67 sec.

Swelling Index

It is determined by measuring the change in dimensions or weight of a material before and after it has absorbed the liquid. The calculation is typically expressed as a percentage, representing the percentage increase in size or weight. The swelling index of RG-CR layer was found to be 60% (n=3) Table 5. A swelling index of 60 means that the material has the ability to absorb and swell up to 60% of its original size or weight when exposed to the specific liquid as shown in Figure 4. A higher swelling index indicates a greater extent of swelling. In this case, the value of 60 suggests that the GR-FBT formulation exhibits a significant swelling response. Both Eudragit RSPO and HPMC K100M can affect the swelling index of a material, but in different ways. Eudragit RSPO is insoluble in water but swells and becomes permeable when exposed to an aqueous medium. Increasing the concentration of Eudragit RSPO in a formulation can generally lead to an increase in the swelling index. HPMC-K100M is a hydrophilic polymer exhibit gel-forming properties and swells upon contact with water, forming a gel-like structure. Both Eudragit RSPO and HPMC-K100M contribute in the swelling property of the RG-CR layer.

In-vitro floating studies

The in-vitro floating studies provide information about the floating behaviour of the GR-FBT formulation (Table 5), GR-FBT is made to float in the stomach for a considerable period of time. It was found that the GR-FBT's floating lag time and total floating time were 4.25±0.32 (n = 3) and more than 24 hours (n = 3), respectively.

In-vitro drug release study

In-vitro drug release test was performed for GR-FBT using USP Type II dissolution apparatus (Electrolab, India). Both MH layer and RG layer dissolution studies was performed. Drug samples for MH layer were taken for at 0, 0.25, 0.5, 0.75 and 1h and drug samples for RG layer were taken at : 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 h. The cumulative % drug release of MH-IR layer as shown in Figure 5 was found to be 81.02%, 93.15%, 99.14%, and 100% at 0.25, 0.5, 0.75 and 1h respectively. More than 85% drug was released within 0.5h. The dissolution profile of MH shows an increase in concentration over time, with a rapid dissolution rate initially and a slower rate as the dissolution progresses. The maximum dissolution is achieved around 45-50 minutes, after which the concentration plateaus. The cumulative % drug release of RG-IR layer was found to be 27.01%, 68.51%, and 92.45% at 1.5, 8, and 24 hour respectively. After 0.25 hours, the concentration of RG increases to 7.32%. This indicates that some dissolution has occurred within this timeframe. As time progresses, the concentration of RG continues to increase. At 0.5 hours, the concentration is 12.54%, and at 0.75 hours, it further rises to 18.41%. After 1 hour, the concentration of RG reaches 21.54%, indicating a steady increase in dissolution. The dissolution rate appears to slow down slightly between 1.5 hours and 2 hours, with the concentration increasing to 27.01% at 1.5 hours and 30.11 at 2 hours. As time progresses, the dissolution rate increases again and at 8 hours, it reaches 68.51%. Finally, after 24 hours, the concentration of RG reaches 92.45%. This suggests that the dissolution of RG continues but at a much slower rate compared to earlier time points. In summary, the dissolution profile of RG shows an increase in concentration over time, with a relatively rapid dissolution rate initially, followed by a slower rate as time progresses. The dissolution appears to continue for at least 24 hours, indicating a controlled release of RG over an extended period.

Stability study

The drug content and physical appearance of the GR-FBT were examined as a result of stability experiments to determine the effects of temperature and relative humidity (RH). According to ICH regulations, the GR-FBT underwent stability studies (mean±SD) (n = 3) for six months while being stored at 25±2^{0}C / 60±5% RH and 40±2^{0}C / 75±5% RH. The GR-FBT's external appearance was unaltered throughout the investigation under all stability circumstances as shown in Table 6. The drug content of the GR-FBT was obtained in the range of 98.41 to 99.47 for RG-IR layer and 98.32 to 99.65 for MH-IR layer respectively for six months; hence, the GR-FBT was considered to be stable.

***In-vivo* study**

***In-vivo* gastro-retentive study**

To evaluate the stomach retention feature of the GR-FBT, an X-ray imaging experiment was carried out on Albino rabbits. Using an X-ray machine, X-ray images of rabbits were taken at 2nd, 8th and 24th hours as shown in Figure 6. This study reveals confirmation that GR-FBT floated on gastric fluid, undergoes continuous swelling and remained in the stomach for more than 24h.

Determination of pharmacokinetic parameters

The figure 7 and 8 depict the plasma concentration-time profiles of MH and RG following the administration of both the Reference Conventional Tablet (RCT) and the Gastro-retentive Floating Bilayer Tablets (GR-FBT) in albino rabbits. Detailed information regarding the pharmacokinetic parameters of the two drugs can be found in the accompanying Table 8. The maximum observed concentration (C_{max}) of MH was nearly similar between the RCT (17.68 µg/ml) and GR-FBT (17.92 µg/ml) formulations. This suggests that both formulations achieve comparable peak concentrations of MH in the bloodstream. The T_{max} of MH was identical for both the RCT (1.5 hours) and GR-FBT (1.5 hours) formulations. This indicates that MH was absorbed at a similar rate from both formulations. The elimination half-life ($T_{1/2}$) of MH was comparable between the RCT (2.52 hours) and GR-FBT (2.68 hours) formulations. This suggests that the elimination rate of MH from the body was nearly similar for both formulations. The mean residence time (MRT) of MH was also nearly similar between the RCT (4.6 hours) and GR-FBT (4.77 hours) formulations. This indicates that MH remains in the body for a similar duration of time for both formulations. This suggests that the time span of MH in the body is comparable for both formulations. The $AUC_{(0-t)}$ for MH is slightly higher in the GR-FBT formulation (96.88 µg/mL h) compared to the RCT formulation (93.15 µg/mL h). This suggests that overall exposure to MH is slightly greater with the GR-FBT formulation. The pharmacokinetic parameters of the MH drug between the RCT and GR-FBT formulations when compared, there are generally no significant differences because MH in both formulation are as immediate release layer. The C_{max}, T_{max}, $T_{1/2}$, and MRT were similar, indicating comparable absorption, elimination, and residence times. However, the $AUC_{(0-t)}$ is slightly higher for the GR-FBT formulation, suggesting slightly greater overall exposure to MH with that formulation.

C_{max} of RG was lower in the GR-FBT formulation (0.1936 µg/ml) compared to the RCT formulation (0.428 µg/ml). This suggests that the GR-FBT formulation leads to a lower peak concentration of RG in the bloodstream compared to the RCT formulation. T_{max} of RG is significantly longer in the GR-FBT formulation (6 hours) compared to the RCT formulation (1 hours). This indicates that RG is absorbed at a slower rate

from the GR-FBT formulation, leading to a delayed Tmax. The elimination half-life ($T_{1/2}$) of RG was significantly longer in the GR-FBT formulation (8.61 hours) compared to the RCT formulation (2.65 hours). This suggests that RG is eliminated from the body at a slower rate when administered through the GR-FBT formulation. The MRT of RG was considerably longer in the GR-FBT formulation (14.33 hours) compared to the RCT formulation (3.94 hours). This indicates that RG remains in the body for a significantly longer duration when administered through the GR-FBT formulation. $AUC_{(0-t)}$ for RG was higher in the GR-FBT formulation (1.99 µg/mL h) compared to the RCT formulation (1.45 µg/mL h). This suggests that overall exposure to RG was greater with the GR-FBT formulation. In summary, when comparing the pharmacokinetic parameters of the RG drug between the RCT and GR-FBT formulations, significant differences were observed. The GR-FBT formulation results in lower C_{max}, delayed T_{max}, longer $T_{1/2}$, extended MRT, and slightly higher $AUC_{(0-t)}$ compared to the RCT formulation. These differences indicate slower absorption, prolonged presence, and increased exposure to RG when administered through the GR-FBT formulation. The study demonstrated that the GR-FBT effectively enhanced the bioavailability of RG in rabbits. The GR-FBT exhibited greater bioavailability compared to RCT likely due to the extended gastric residence time resulting from the tablet's flotation in the stomach. The Independent Sample t-Test was conducted using SPSS software to compare the MH (IR) RCT and MH (IR) GR-FBT, yielding an insignificant result (P=0.981) (P>0.05). Similarly, the Independent Sample t-Test was performed to compare the RG (IR) RCT and RG (CR) GR-FBT, revealing a significant finding (P=0.003) (P<0.05).

***In-vitro-in vivo* correlation studies [25]**

Since the values of the correlation coefficient (R) of RG were greater than 0.9 (Figure 9).This suggests that the in vivo performance could be accurately predicted by the test of in vitro release.

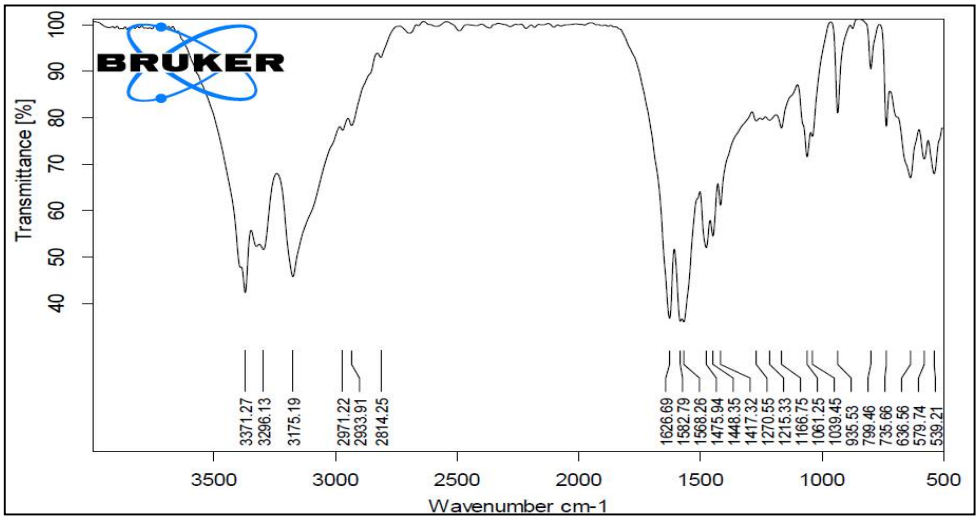

Figure 1: FTIR spectra of Pure MH and RG (1:1 mixture)

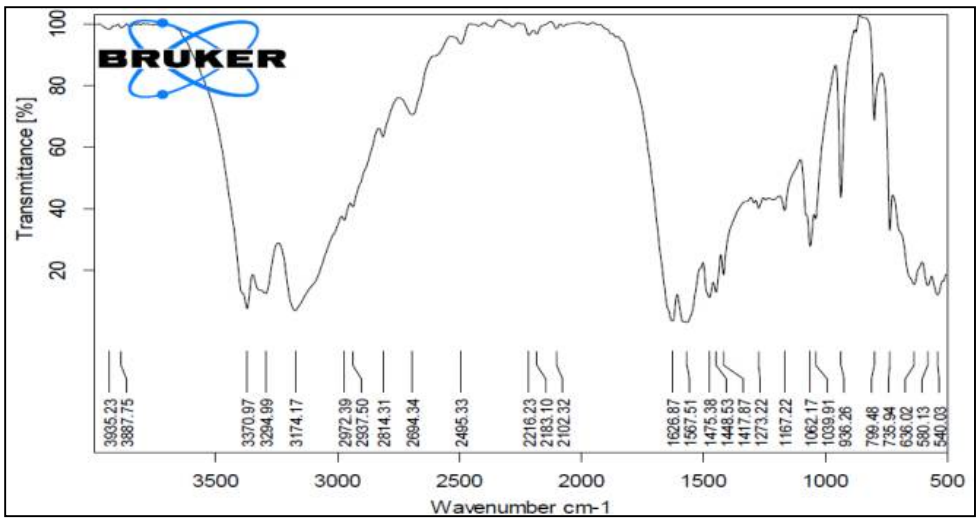

Figure 2: FTIR spectra of Pure MH and Mixture (polymer and excipients)

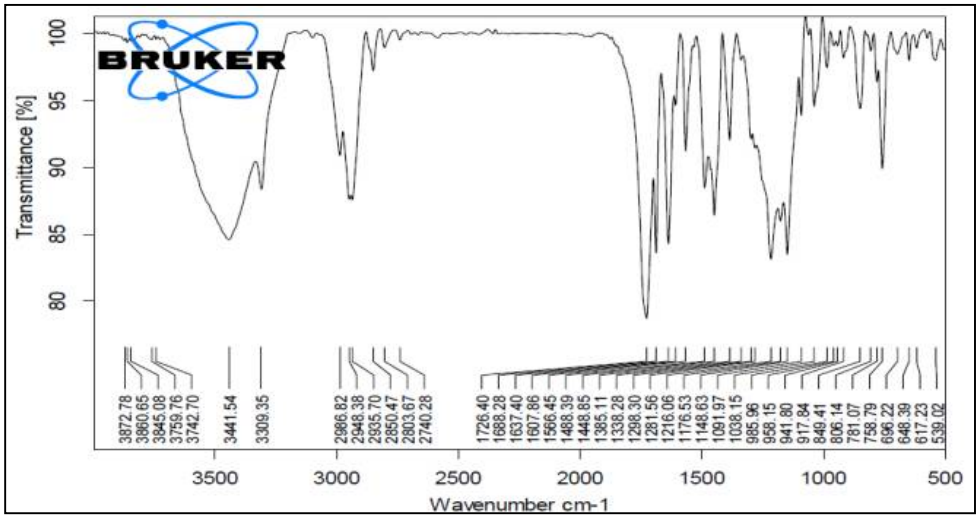

Figure 3: FTIR spectra of Pure RG and Mixture (polymer and excipients)

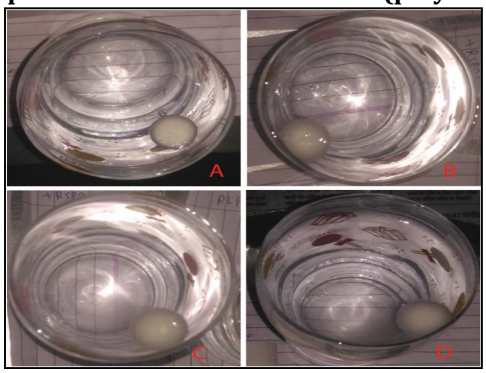

Figure 4: The swelling extent of the GR-FBT at A) 1.5h B) 4h C) 8h and D) 24h

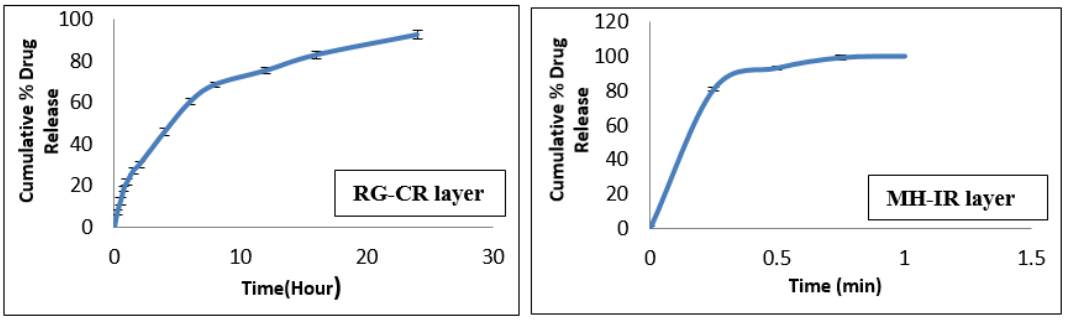

Figure 5: Dissolution profile of RG-CR and MH-IR layer

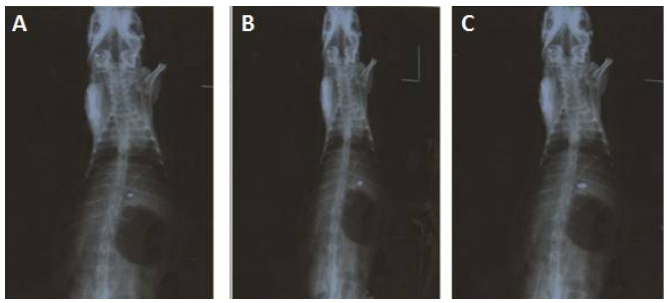

Figure 6: X-ray photographs of GR-FBT in a rabbit (A) 2h (B) 8h (C) 24h

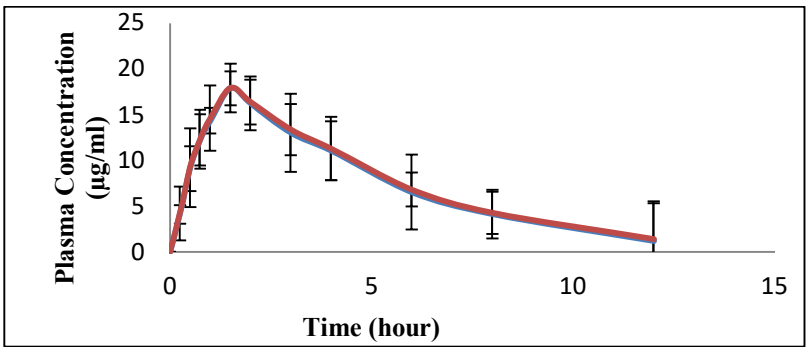

Figure 7: Plasma concentration as a function of time after single oral dose of 1.01 mg/kg of MH from CRT and GR-FBT (n=3)

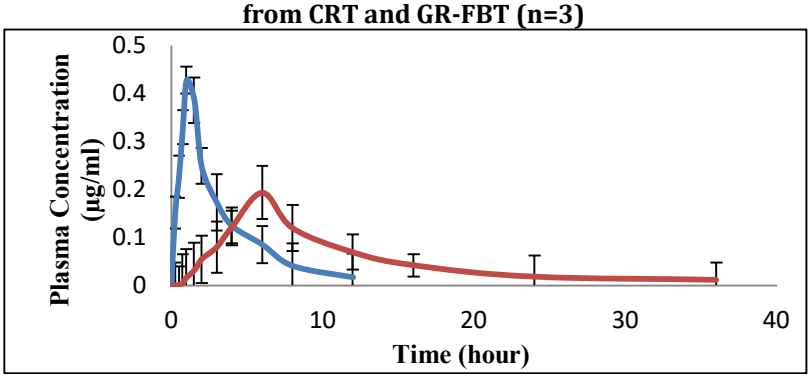

Figure 8: Plasma concentration as a function of time after single oral dose of 0.09 mg/kg of RG from RG (RCT) and RG (GR-FBT) (n=3)

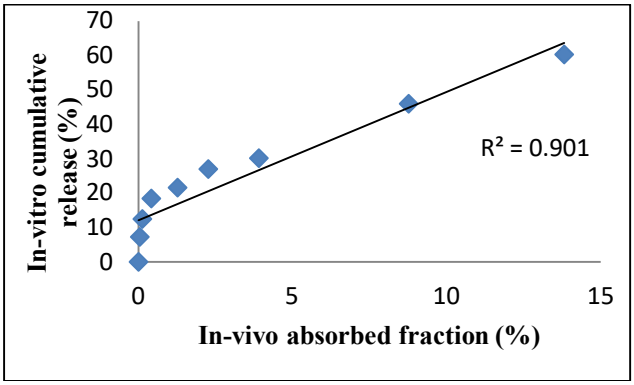

Figure 9: Plots of the percentage of the dose absorbed (RG) versus the mean percentage of the dose released (RG) from the GR-FBT.

Table 1: Preliminary trial batches with observations

S. No.	Polymer	Observations
1	Precirol ATO 5 and HPMC K-4M	Not float
2	Precirol ATO 5 and HPMC E-50 LV	Not float
3	Precirol ATO 5 and Carbopol 934	Not float and tablet break
4	Precirol ATO 5 and HPMC E-5 LV	Tablet intact but Not float
5	Eudragit RSPO and Carbopol 934	Float but whole tablet dissolve in 12 h
6	Eudragit RSPO and HPMC K-100M	Float up to 26 h

Table 2: Composition of Metformin Hydrochloride immediate release layer (MH-IR)

Quantity (mg) / Formulation	MH	Crospovidone	croscarmellose sodium	Sodium Starch Glycolate	MCC-101	Magnesium stearate	Talc
MH-1	250	5	-	-	140	2.5	2.5
MH-2	250	10	-	-	135	2.5	2.5
MH-3	250	15	-	-	130	2.5	2.5
MH-4	250	-	5	-	140	2.5	2.5
MH-5	250	-	10	-	135	2.5	2.5
MH-6	250	-	15	-	130	2.5	2.5
MH-7	250	-	-	5	140	2.5	2.5
MH-8	250	-	-	10	135	2.5	2.5
MH-9	250	-	-	15	130	2.5	2.5

Table 3: Post-compression parameters of Metformin Hydrochloride Immediate-release layer (MH-IR)

Formulation Code	Thickness ± SD (mm) (n=10)	Drug Content (%) (n=10)	Weight variation (n=20)	Hardness ± SD (kg/cm^2) (n=5)	Friability (%) (n=10)	Disintegration time (Sec) (n=6)
MH-1	1.53±0.073	99.17±1.45	397±6.12	4.5±0.3	0.319±0.6	48±1.98
MH-2	1.43±0.051	99.57±1.78	412±5.22	4.2±0.2	0.411±0.5	38±1.64
MH-3	1.58±0.082	98.54±2.34	390±4.31	4.9±0.5	0.380±0.6	31±1.24
MH-4	1.48±0.060	98.87±1.74	392±5.88	4.7±0.4	0.389±0.4	50±1.98
MH-5	1.53±0.045	99.12±1.61	403±6.41	4.3±0.3	0.410±0.5	43±1.98
MH-6	1.40±0.041	98.41±2.61	417±4.68	4.4±0.5	0.218±0.4	40±1.98
MH-7	1.51±0.061	99.04±2.31	396±5.32	4.1±0.4	0.384±0.4	54±1.98
MH-8	1.46±0.041	98.31±2.18	411±4.55	4.6±0.4	0.412±0.5	46±1.98
MH-9	1.47±0.051	98.15±1.27	394±6.24	4.3±0.3	0.440±0.4	38±1.98

Table 4: Composition of Gastro-retentive (Floating) Bilayer tablets (GR-FBT)

MH-Immediate Release Layer (400mg)	
Excipients	Quantities (mg)
MH	250
Crospovidone	10
MCC-101	135
Magnesium stearate	2.5
Talc	2.5
RG-Controlled Release Layer (400mg)	
RG-SD	22
Eudragit RSPO	14.35
HPMC-K 100M	44.44
NaHCO3	10
Citric Acid	5
Xantham Gum	30
MCC-101	166.22
Lactose	100
Mg-stearate	4
Talc	4

Table 5: Post-compression Characterization of GR-FBT (mean± SD)

S. No.	Parameters	Results
1	Thickness (mm) (n=10)	3.4±0.05
2	Average Weight (n=20) (mg)	784±3.78
3	Hardness (kg/cm^2) (n=5)	7.6±0.61
4	Friability (%) (n=10)	0.354±0.002
5	Drug Content (%) (MH-IR Layer) (n=10)	99.72±2.41
6	Drug Content (%) (RG-CR Layer) (n=10)	99.16±3.21
7	Disintegration time (MH-IR Layer) (in sec) (n=6)	33±1.67
8	Floating lag time (RG-CR Layer) (in min) (n=3)	4.25±0.32
9	Swelling Index (CR Layer) (%) (n=3)	60±0.44
10	Total Floating Time (CR Layer) (n=3)	>24

Table 6: Result of Stability studies

Stability Condition	Sampling Interval (Months)	Physical Appearance	RG-CR layer Drug Content (%)	MH-IR layer Drug Content (%)
25 ± 2 ^{0}C / 60 ± 5% RH	0	Good	99.47±0.07	99.65±0.06
	3	No change	98.43±0.08	99.61±0.07
	6	No change	98..41±0.06	98.32±0.06
40 ± 2 ^{0}C / 75 ± 5% RH	0	Good	99.37±0.07	99.28±0.07
	3	No change	98.78±0.06	99.23±0.05
	6	No change	98.65±0.06	98.87±0.06

Table 7: In-vivo pharmacokinetic study protocol for GR-FBT

Animal Group	No. of Animals	Formulation
Group A	3	GR-FBT
Group B	3	Reference conventional tablet (RCT)

Table 8: Pharmacokinetic Parameters of MH and RG after administration of RCT and GR-FBT) in Rabbits (n=3)

Formulation		Cmax (µg/ml)	Tmax (h)	T1/2 (h)	MRT (h)	AUC(0–t) (µg/mL h)
RCT	MH	17.68±1.26	1.5±0.12	2.52±0.31	4.6±0.15	93.15±6.23
	RG	0.428±0.11	1±0.17	2.65±0.22	3.94±0.23	1.45±0.38
GR-FBT	MH	17.92±1.48	1.5±0.11	2.68±0.13	4.77±0.36	96.88±2.45
	RG	0.1936±0.011	6±0.44	8.61±0.67	14.33±0.78	1.99±0.58

CONCLUSION

The current research is carried out to formulate controlled release gastro-retentive floating bilayer tablets containing Metformin Hydrochloride as immediate release layer and Repaglinide as a controlled release layer into two separate layers using computer aided optimization that will release the drug continuously for more than 24 hours. The bilayer tablets exhibited excellent buoyancy, remaining afloat on the test medium for over 24 hours. The tablets showed a floating lag time of 4.25 minutes. In vivo studies conducted on rabbit's revealed favourable absorption of Repaglinide from the bilayer tablets, leading to enhanced bioavailability. The pharmacokinetic analysis showed significantly lower C_{max} and significantly higher T_{max} values for Repaglinide in comparison to the reference conventional tablet. Furthermore, the bilayer tablets exhibited a significantly prolonged mean residence time and half-life, indicating controlled drug release and prolonged drug action. Further evidence that the in vitro release data may be used as an accurate predictor of drug absorption came from the in vitro drug release profile's significant connection with in vivo drug absorption. The dual drug release properties were intended for the optimised bilayer tablets. Repaglinide has a regulated release pattern, whereas Metformin Hydrochloride was designed for rapid release. In the treatment of Type 2 diabetes, this combination of release patterns enables controlled therapeutic effects, improving health outcomes. The formation of this GR-FBT is a cutting-edge and optimistic method for controlling Type 2 diabetes, with potential advantages for patients.

CONFLICTOF INTEREST

Declared none

ETHICAL APPROVAL

The approval for animal studies was taken from the Institutional Animal Ethical Committee (IAEC) of Adina Institute of Pharmaceutical Science, Sagar. The IAEC number assigned to the study is 1546/PO/E/S/11/CPCSEA.

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