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Synchronized and controlled release of metformin hydrochloride/glipizide from elementary osmotic delivery

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Abstract

The combination of metformin hydrochloride (MTF) and glipizide (GLZ) is second-line medication for diabetes mellitus type 2 (DMT2). In the present study, elementary osmotic pump (EOP) tablet is designed to deliver the combination of MTF and GLZ in a sustained and synchronized manner. By analyzing different variables of the formulation, sodium hydrogen carbonate is introduced as pH modifier to improve the release of GLZ, while ethyl cellulose acts as release retardant to reduce the burst release phase of MTF. A two-factor, three-level face-centered central composite design (FCCD) is applied to investigate the impact of different factors on drug release profile. Compared with conventional tablets, the EOP tablet demonstrates a controlled release behavior with relative bioavailability of 99.2% for MTF and 99.3% for GLZ. Data also shows EOP tablet is able to release MTF and GLZ in a synchronized and sustained manner both in vitro and in vivo.

Keywords: Elementary osmotic pump (EOP); face-centered central composite design; metformin hydrochloride/glipizide; synchronized and sustained release.
Introduction

Diabetes mellitus type 2 (DMT2) is a metabolic disease characterized by insulin resistance and deficiency with high blood glucose level, which also referred as non-insulin dependent diabetes\(^1\). Increased thirst, frequent urination and constant hunger are usually accompanied with the onset of DMT2, followed by a series of complications if DMT2 is improperly treated\(^2\). Physical exercise and healthy diet are considered to be pivotal to treat DMT2 at first\(^3,4\), however medication is required to control blood glucose level if the disease deteriorates. According to international diabetes federation, more than 8% of the world population suffer from DMT2 and this number is expected to rise in the next two decades\(^5\). Consequently, stable and effective medicine is in urgent needed for the treatment of DMT2.

Anti-diabetic drugs aim at maintaining a normal blood glucose level by reducing plasma glucose concentration. Compared with injectable insulin formulation, oral anti-diabetic drugs are increasingly in favor of physicians due to their ease of use with better control of blood glucose level\(^6-8\). Research has shown the mechanism of anti-diabetic drugs is either by improving the output and sensitivity of insulin itself, such as sulfonylurea, or regulating blood glucose absorption thereby maintaining a normal blood glucose level\(^9,10\). Biguanide and sulfonylurea is considered the second-line anti-diabetic drugs due to their relatively high bioavailability and marginal side effect. As one of biguanide derivatives, metformin hydrochloride (MTF) decreases blood glucose level by the inhibition of hepatic glucose production. Alternatively, as one of sulfonylurea derivatives, glipizide (GLZ) acts directly in pancreatic islet \(\beta\)-cells to facilitate the secretion of insulin\(^6,11\). The combination of MTF and GLZ is recommended by many physicians due to their complimentary effects in decreasing
blood glucose level in different mechanisms\textsuperscript{12,13}. This complimentary effect represents one of the advantages in the combination of MTF and GLZ. Metaglip\textsuperscript{TM} (MTF and GLZ Tablets, Bristol-Myers Squibb, US) is very popular in the diabetics worldwide. However, the fluctuation of blood glucose concentration caused by traditional fast release preparation could induce serious side effects. Hence, the sustained release anti-diabetic agents attract so much attention of researchers. Because they could maintain a steady blood drug level and reduce dosage strength and dosing frequency\textsuperscript{14}. Among these sustained drug delivery systems, osmotic pump system is much more superior to others because of its more stable blood drug level, better \textit{in vitro} and \textit{in vivo} correlation and free from the influence of physiological factors like pH and gastrointestinal peristalsis\textsuperscript{15}.

Recently, osmotic pump system has made a substantial progress in the delivery of different drugs with varied water solubility\textsuperscript{16}. Apart from chemical drugs, many emulsions, nanoparticles, traditional Chinese medicines and compound medicines could also be delivered by this technology. Lanlan Wei \textit{et al.} reported a novel self-emulsion carvedilol elementary osmotic pump\textsuperscript{17}, Xi Zhang \textit{et al.} have investigated the controlled release of a cyclosporine self-nanoemulsifying preparation through osmotic pump technology\textsuperscript{18}, Dandan Liu \textit{et al.} studied the delivery of carvedilol nanosuspension through an osmotic pump capsule\textsuperscript{19}. The intention of this design is to take advantage of the merits of emulsion and nanoparticle—improving drug absorption and bioavailability, meanwhile controlling drug release and maintain blood drug level. The osmotic pump preparation of traditional Chinese medicines and compound medicines could make good use of the synergism of different drugs and reduce the fluctuation of blood drug concentration\textsuperscript{20,21}. 
Hence, considering the connection of MTF and GLZ and sustained drug release, we investigated MTF and GLZ elementary osmotic pump (EOP). Generally, EOP is only suited to the drug having high water solubility like MTF, and not suitable for drugs with low solubility like GLZ\textsuperscript{15, 22}. Because EOP could not offer sufficient driving force for insoluble drug to reach complete drug release. However, In terms of the EOP system of MTF and GLZ, MTF could act as an osmotic agent which generates powerful osmotic pressure to facilitate the release of GLZ, which has been proved to be true in many investigations\textsuperscript{23, 24}. Therefore, the sustained and synchronized release profiles of MTF and GLZ are achieved by the employment of EOP system.

In the present study, we establish an EOP formulation of MTF and GLZ with sustained and synchronized release profile to realize synergistic effect of the two drugs and maintain stable, prolonged drug level. Formulation variables are investigated by a number of factors, including tablet strength and membrane coating thickness\textsuperscript{25}. A 2-factor, 3-level face-centered central composite design (FCCD) is applied to optimize the formulation\textsuperscript{26, 27}. Mathematical and graphical models are also implemented to study the impact of variables on release profiles. At last, the pharmacokinetics study of the optimized EOP tablet is performed in beagle dogs.

**Materials and Methods**

**Materials**

Metformin hydrochloride was purchased from Jiameng Pharmaceutical Co. Ltd. (Anhui, China). Glipizide was a gift sample from Sciecure Pharmaceutical Co. Ltd. (Beijing, China). Plasdone\textsuperscript{®} K-90 (PVP K-90) was a gift sample from ISP Technologies Inc. (New Jersey,
USA). Ethyl cellulose (EC), sodium hydrogen carbonate and magnesium stearate were purchased from Bodi Chemical Co. Ltd. (Tianjin, China). Cellulose acetate (CA) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Polyethylene glycol (PEG-400, 1500, 4000; the number is the molecular weight of PEG) was purchased from Kernel Chemical Reagent Co. Ltd. (Tianjin, China). Metformin hydrochloride and glipizide tablets were purchased from Lifeon Pharmaceutical Co. Ltd. (Anhui, China). All other ingredients were in analytical grade.

**Methods**

**Preparation of core tablet**

MTF, GLZ, PVP K-90, EC and sodium hydrogen carbonate were passed through sieve No. 80 (opening size, 180 µm) separately. Drugs and all the other ingredients were weighed by balance and mixed in mortar. Granules were prepared by wet granulation using 95% alcohol as a moistening agent and passed through sieve No. 20 (opening size, 850 µm). The granules were dried at 40 °C for 2 h and passed through sieve No. 18 (opening size, 1000 µm). Magnesium stearate was blended with dry granules and compressed into tablets using a single station punching machine (Shanghai No. 1 Pharmaceutical Device Co., Shanghai, China) fitted with 11 mm concave punches.

**Coating of core tablet**

The osmotic pump tablets were prepared with a semi-permeable membrane to obtain the desired release profile. Coating solution was prepared by dissolving CA and PEG in a solution of acetone and water (95:5, v/v). Core tablets were placed in the coating pan (Shanghai Tianfan Machinery Factory, Shanghai, China) along with 100 g placebo tablets.
Pan-rotating rate was 35 rpm, spray rate was 6 mL/min, and drying temperature was 30 °C. Coating process continued until desired weight was achieved on tablet core. The coated tablets were dried overnight at 40 ºC to remove the residual solvent.

**In vitro dissolution study**

In vitro dissolution study was performed using USP II (paddle) apparatus (ZRS-6G, Tianjin Tianda Tianfa Technology Co. Ltd., Tianjin, China). A 0.05 M pH 6.8 phosphate buffer of 1000 ml was used as the dissolution medium maintained at 37 ± 0.5 ºC) at a rotation speed of 50 rpm. 5 ml samples were withdrawn from the dissolution medium at 0, 2, 4, 6, 8, 10, and 12 h and filtered through 0.45 μm cellulose nitrate filters in 30 seconds. Each study was performed in triplicate and the mean values were recorded accordingly.

**Determination of MTF:**

The filtrated sample was diluted with pH 6.8 phosphate buffer (dissolution medium) and determined at 233 nm by UV spectrophotometric (T6, Beijing Purkinje General Instrument Co., Ltd., Beijing, China).

**Determination of GLZ:**

The filtrated sample was analyzed by HPLC (L6-P6, Beijing Purkinje General Instrument Co. Ltd., Beijing, China). The separation of GLZ in dissolution sample was performed on a Diamonsil C18 column (5 μm, 200 × 4.6 mm, Dikma). Mobile phase was consisted of 0.025 M pH 6.0 potassium dihydrogen phosphate buffer and methanol (40:60, v/v). The mobile phase was pumped at a flow rate of 1 ml/min. The wavelength of UV detector was set at 225 nm. The injection volume was 20 μl.

**Comparison of in vitro release profile**
The method of similarity factor ($f_2$) was recommended by the Food and Drug Administration (FDA) for dissolution profile comparison $^{31,32}$. Two dissolution profiles were considered to be similar when the value of $f_2$ was between 50 and 100. The $f_2$ was calculated using the following equation:

$$f_2 = 50\log\left\{1 + \frac{1}{n} \sum_{i=1}^{n} (R_t - T_t)^2 \right\}^{-0.5} \times 100 \tag{1}$$

where $n$ was the number of time points, $R_t$ was the dissolution value of the reference profile at time point $t$ and $T_t$ was the test profile at the same time point. The equation was applied to the evaluation of differences between the formulations. $R_t$ and $T_t$ were replaced with the dissolution value of the two formulations, respectively.

**Design of EOP tablets**

As described in Table 1, different formulations were designed to study factors influencing drug release profile. For example, different coating materials were used to study the effect of pore-forming agent on drug release.

**Optimization of EOP tablet**

In order to optimize the formulation of EOP tablet, a 2-factor, 3-level face-centered central composite design was applied in this study. Each factor was consisted of three groups of design points: the points of the full factorial design stayed at the factor level of $-1$ and $+1$; the points of the star design stayed at the levels of $0$, $-\alpha$ and $+\alpha$; and the center point stayed at the factor level of $0$ $^{27,33}$. Compared with circumscribed central composite design, FCCD evaluated the factors at three levels with $\alpha = 1$ (Table 2). Thus, the experimental trails were composed of 9 possible combinations, including 4 factorial points, 4 axial points and 5
Moreover, two independent variables (factors): CA: PEG-1500 ratio ($X_1$) and weight gain ($X_2$) were selected to study their effects on the release profile of the two drugs. The EOP tablet was designed to release drugs in 12 h with zero-order release rate. Thus, four dependent variables (responses): percentage of MTF released within 12 h ($Q_{MTF\_12\_h}$, $Y_1$), $R^2$ of MTF release data fitted to zero-order equation ($RSQ_{MTF\_zero}$, $Y_2$), percentage of GLZ released within 12 h ($Q_{GLZ\_12\_h}$, $Y_3$), and $R^2$ of GLZ release data fitted to zero-order equation ($RSQ_{GLZ\_zero}$, $Y_4$) were selected to evaluate the release profiles. All experiments were performed in triplicate and randomized manner to eliminate a possible source of bias.

The statistical experimental design was performed for model qualification. The regression coefficients were determined by the Design-Expert software (Version 8.0.5, Stat-Ease Inc., Minneapolis, USA).

**In vivo study in beagle dogs**

The protocol of *in vivo* study was approved by the university ethics committee under the guidance for care and use of laboratory animals. The *in vivo* study was performed in the department of laboratory animal research at Shenyang Pharmaceutical University (Shenyang, China).

A randomized, two-period crossover design was conducted to evaluate *in vivo* performance of EOP tablet. Six healthy beagle dogs, weighing between 9 and 13 kg, were used in this study. The dogs were kept overnight fasting for at least 12 h prior to experiment with free access to water. All dogs were divided into two groups. One group was given two conventional tablets (each tablet contains 250 mg MTF with 2.5 mg GLZ), whereas the other
group was given one EOP tablet (containing 500 mg MTF with 5 mg GLZ). All formulations were administrated to dogs with 20 ml of water. A washout period of at least 7 days was required between two consecutive administrations.

5 ml blood samples were obtained from cephalic vein at certain time points after administration. All blood samples were kept in heparinized tubes, and immediately centrifuged at 4000 rpm for 10 min. The plasma was removed and stored at −20 °C for further analysis.

Sample preparation and analytical method

Determination of plasma MTF concentration:

0.2 ml plasma was added with 0.4 ml methanol before vortex for 1 min. The plasma was centrifuged at 12,000 rpm for 10 min. 20 μL of supernatant was directly injected into the column for HPLC analyses under the conditions describe below.

The concentration of MTF in the blood sample was analyzed by HPLC (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). The separation of MTF was achieved on a Diamonsil C18 column (5 μm, 250 × 4.6 mm, Dikma). The mobile phase consisted of 2 mm sodium dodecyl sulfate solution (0.25% (v/v) triethylamine, pH 3.6) and acetonitrile (64:36, v/v), and flow rate was 1.0 ml/min. The wavelength of UV detector was set at 233 nm.

The injection volume was 20 μl.

Determination of plasma GLZ concentration:

0.5 ml plasma was added with 50 μl methanol solution of gliclazide (10 μg/ml) as internal standard. Then the plasma was added with 200 μl 0.4 M HCl before vortex for 30 s. Vortex the plasma for another 10 min with 3 ml diethyl ether. Then the plasma was centrifuged at
4,000 rpm for 5 min. The supernatant was removed and dried at 45 °C by nitrogen. The residue was subsequently reconstituted with 100 μl methanol and analyzed by HPLC.

The concentration of GLZ in the blood sample was analyzed by HPLC35 (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). The separation of GLZ was achieved on a Diamonsil C18 column (5 μm, 200 × 4.6 mm, Dikma). The mobile phase consisted of water (0.1% (v/v) acetic acid, pH 3.4), acetonitrile, and methanol (55:35:10, v/v/v), and flow rate was 1.0 ml/min. The wavelength of UV detector was set at 225 nm. The injection volume was 20 μl.

Data analysis and statistics

Data were analyzed by DAS 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The maximum plasma concentration (C_max) and time to reach the maximum plasma concentration (T_max) were obtained directly from the curve. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule. AUC and C_max were log-transformed prior to analysis with t-test. T_max was analyzed using nonparametric Wilcoxon test. Difference was considered significant with p value < 0.05.

The relative bioavailability of test preparation was determined by the ratio of the test preparation AUC to the reference preparation AUC. The preparations were considered bioequivalent if the ratio stayed within the range of 80-125%.

The relationship between in vitro cumulative release and the fraction of drug absorbed in vivo was established with in vitro and in vivo correlation (IVIVC) and coefficient correlation (R).

Result and Discussion
Design of EOP tablet and the effect of different factors in relation with release profile

Drug release profile of the initial formulation

The initial formulation is established on the basis of a previous formulation with the expectation of sustained and synchronized release of MTF and GLZ (Table 1). Fig. 1 illustrates the drug release profile of the initial formulation; the cumulative release of MTF in 12 h is 83.2%, whereas the cumulative release of GLZ in 12 h is 25.0%. A burst release phase lasts from 4 h to 6 h. Compared with MTF, the release rate of GLZ is relatively low with less cumulative release of the drug in 12 h.

Effect of pH levels on drug release

GLZ is insoluble in water with pKₐ at 5.9. In order to deliver GLZ in a sustained release manner, sufficient osmotic pressure plays an important role. More importantly, osmotic pressure is crucial in the preparation of EOP tablet especially for a poorly water-soluble drug 36-38, such as GLZ. Therefore, high dose of MTF in the core tablet is used as an osmotic active agent to generate sufficient osmotic pressure for controlled release of GLZ. In this article, the solubility of GLZ varies with different pH levels. Fig. 2a-b (F01-F03) shows the impact of NaHCO₃ on the release profile of the formulation. The release rate of GLZ is higher as the concentration of NaHCO₃ rises. As a pH modifier, NaHCO₃ changes pH of the solution in the tablet core, which eventually leads to higher solubility of GLZ 39,40. With the help of high dose of MTF and pH modifier, cumulative release of GLZ in 12 h improves more than threefold compared with the initial formulation 41.

Effect of release retardant on drug release

The high water-solubility of MTF comes with problem of burst release phase in a certain
formulation, resulting in difficulties in the control of drug release rate\textsuperscript{42}. As an impermeable polymer, ethyl cellulose (EC) is one of the materials with the capability to address this issue\textsuperscript{43,44}. In this study, EC is added to the formulation as both binder and release retardant. Fig. 2a-b (F04-F06 and F07-F09) shows the release profiles of the formulation with different moistening agents. No burst release is observed from 4h to 6h and release profile is unaffected by different amounts of EC.

**Effect of pore-forming agent on drug release**

Fig. 2c-d (F10-F12 and F13-F15) shows the impact of different pore-forming agents, such as PEG, on the release profile of the formulation. PEG works by forming more pores on the membrane of the tablet, which leads to higher release rate of the drug\textsuperscript{45}. In this study, the release profiles of PEG-400, PEG-1500 and PEG-4000 are similar, whereas the release curves are significantly influenced PEG levels. As shown in the figures, the drug release rate (F13-F15) and cumulative release of both MTF and GLZ in 4 h increase when the PEG-1500 level increases.

**Effect of membrane coating weight gain on drug release**

Fig. 2c-d (F16-F18) shows the impact of coating weight gain on the release profile of the formulations. It is observed that drug release rate and cumulative release decreases from F16 to F18 for both MTF and GLZ. The result shows that the drug release rate decreases as the coating weight gain increases. When because coating weight gain decrease, water penetration across the membrane increase. Hence, tablet core is dissolved faster, and the release rate ascends.

**Optimization of EOP tablet**
The traditional one-variable-at-a-time (OVAT) formulation optimization is in search of an optimal response from one certain variable by keeping all the other factors in fixed level. Design of Experiment (DoE) triumphs OVAT by improving interactions between factors. In our study, a two-factor, three-level face-centered central composite design (FCCD) is used for the optimal response of different factors in relation with the formulation. All factors are intentionally divided into two groups, the first group contains the factors in relation with core tablet, while the other group contains the factors affecting the property of the semi-permeable membrane. CA: PEG-1500 ratio and membrane coating thickness are selected for formulation optimization. By the calculation of design expert software, 13 possible formulations are generated (Table 3). In particular, F07 is selected as the optimal formulation for the core tablet.

**Statistical analysis and mathematical modeling**

The effect of independent parameters CA: PEG-1500 ratio \(X_1\) and weight gain \(X_2\) in responses to \(Q_{MTF \, 12\, h}\) \((Y_1)\), \(RSQ_{MTF \, zero}\) \((Y_2)\), \(Q_{GLZ \, 12\, h}\) \((Y_3)\), and \(RSQ_{GLZ \, zero}\) \((Y_4)\) are analyzed \((Y_1\) and \(Y_3\) are drug cumulative release percentage, while \(Y_2\) and \(Y_4\) are \(R^2\) of drug release data fitted to zero-order equation). The mathematical model for each response is generated and visualized by 3D model graph. The relationship between explanatory variables and responses are analyzed by multiple linear regression with better-fitting method which are shown in Eqs. (3) - (6) below.

\[
Y_1 = -68.16516 + 53.57930 \times X_1 + 34.74313 \times X_2 - 4.59750 \times X_1 \times X_2 - 4.64414 \times X_1^2 \\
- 2.62414 \times X_2^2
\]

(3)
\[ Y_2 = +0.24732 \times X_1 + 0.14913 \times X_2 - 0.017050 \times X_1 \times X_2 - 6.66897 \]
\[ - 0.003 \times X_1^2 - 8.11897 \times X_2 - 0.003 \times X_2^2 \]  
(4)

\[ Y_3 = +53.82375 + 18.96708 \times X_1 + 8.47917 \times X_2 - 2.35250 \times X_1 \times X_2 - 1.58000 \times X_1^2 \]
\[ - 0.19000 \times X_2^2 \]  
(5)

\[ Y_4 = +0.041355 + 0.21522 \times X_1 + 0.16811 \times X_2 - 0.019575 \times X_1 \times X_2 - 0.011926 \times X_1^2 \]
\[ - 6.87586 \times X_2 - 0.003 \times X_2^2 \]  
(6)

Eqs. (3)-(6) reflect the quantitative influence of formulation variable: \( X_1 \) (CA:PEG-1500 ratio) and \( X_2 \) (weight gain) and their interaction with response: \( Y_1 \) (Q\(_{\text{MTF 12 h}}\)), \( Y_2 \) (RSQ\(_{\text{MTF zero}}\)), \( Y_3 \) (Q\(_{\text{GLZ 12 h}}\)), and \( Y_4 \) (RSQ\(_{\text{GLZ 12 h}}\)).

By analysis of variance (ANOVA), it indicates the quadratic regression model is suitable for every response \( Y_1 \) (\( p < 0.0001 \)), \( Y_2 \) (\( p < 0.0001 \)), \( Y_3 \) (\( p < 0.0001 \)) and \( Y_4 \) (\( p < 0.0001 \)). Meanwhile, data quality of the model for every response is measured. The value \( R^2 \) indicates the proportion of variance of the model. The \( R^2 \) values of the model are 0.975, 0.982, 0.998 and 0.988 for \( Y_1 \), \( Y_2 \), \( Y_3 \) and \( Y_4 \), which represent 97.5%, 98.2%, 99.8% and 98.8% of the variance for the model. Adjusted \( R^2 \) values for every response \( Y_1 \), \( Y_2 \), \( Y_3 \) and \( Y_4 \) are 0.958, 0.969, 0.996 and 0.979, and the corresponding predicted \( R^2 \) values are 0.851, 0.874, 0.993 and 0.955 (Table 4). The adjusted \( R^2 \) and predicted \( R^2 \) are closer than 0.20, which indicates the predicted \( R^2 \) is in agreement with the adjusted \( R^2 \). The relationship between dependent variables, for example \( Q_{\text{MTF 12 h}} \) (\( Y_1 \)), \( RSQ_{\text{MTF zero}} \) (\( Y_2 \)), \( Q_{\text{GLZ 12 h}} \) (\( Y_3 \)), and \( RSQ_{\text{GLZ zero}} \) (\( Y_4 \)) and independent variables CA: PEG-1500 ratio (\( X_1 \)) weight gain (\( X_2 \)) are demonstrated in Fig. 3a-d. The region of maxima (region in red) and minima (region in blue) for every 4 response
is visualized in the figure as well.

Analysis of MTF release characteristics

CA: PEG-1500 ratio \((X_1)\), weight gain \((X_2)\) and their interaction between \(Q_{MTF\ 12\ h} \ (Y_1)\) and \(RSQ_{MTF\ zero} \ (Y_2)\) are shown in Eqs. (3) and (4).

The regression equation is represented in function using \(x_1\), \(x_2\), and \(f\ (x_1, x2)\) as \(X_1\), \(X_2\), and \(Y\). Eqs. (3) is adapted to the function below.

\[
f(x_1, x_2) = -68.16516 + 53.57930x_1 + 34.74313x_2 - 4.59750x_1 x_2 - 4.64414 x_1^2 - 2.62414x_2^2
\]

\[(7)\]

The partial derivative \(f\) in relation with \(x_1\) and \(x_2\) is calculated, as shown below.

\[
\frac{\partial f}{\partial x_1}(x_1, x_2) = 53.57930 - 4.59750 x_2 - 9.28828 x_1
\]

\[(8)\]

\[
\frac{\partial f}{\partial x_2}(x_1, x_2) = 34.74313 - 4.59750 x_1 - 5.24828 x_2
\]

\[(9)\]

The above two partial derivative functions explain the variation of \(f\) in the \(x_2\) and \(x_1\) direction. Indeed, \(\partial f/\partial x_1\) gives an exact value for every point on the slope in the \(x_1\) direction.

The value range of \(x_1\) in this study is 4 to 6, and that of \(x_2\) is 2.5 to 4.5. Thus, the value range of \(\partial f/\partial x_1\) is an interval from 4.93 to -22.84, and the value range of \(\partial f/\partial x_2\) is an interval from 3.23 to -16.46. The change of partial derivative indicates \(Q_{MTF\ 12\ h} \ (Y_1)\) increases with CA: PEG-1500 ratio \((X_1)\) and weight gain \((X_2)\).

Similarly, Eqs. (4) is established in the same manner. The value range of \(\partial f/\partial x_1\) is an interval from 0.053 to -0.0076, and the value range of \(\partial f/\partial x_2\) is an interval from 0.051 to -0.016. The change of the partial derivative also implies \(RSQ_{MTF\ zero} \ (Y_2)\) increases with CA: PEG-1500 ratio \((X_1)\) and weight gain \((X_2)\). The maximum region is located in the upper
values of both CA: PEG-1500 ratio ($X_1$) and weight gain ($X_2$) where the derivative goes through zero.

Fig. 3a and Fig. 3b also illustrate the quadratic relationship between CA: PEG-1500 ratio and weight gain. An increase in CA: PEG-1500 ratio from 4 to 6 and weight gain from 2.5 to 4.5 results in fall in the graph of $Q_{MTF\,12\,h}$ and rise in the graph of $RSQ_{MTF\,zero}$. Moreover, the graphical analysis is coincident with the mathematical analysis.

**Analysis of GLZ release characteristics**

CA: PEG-1500 ratio ($X_1$), weight gain ($X_2$), the release profile of GLZ in 12 h ($Y_3$) and correlation coefficient ($Y_4$) are illustrated in Eqs. (5) and (6).

The analysis is similar with MTF. In Eqs. (5), the value range of $\frac{\partial f}{\partial x_1}$ is an interval from 0.45 to -10.58, and the value range of $\frac{\partial f}{\partial x_2}$ is an interval from -1.88 to -7.35. The change of partial derivative $\frac{\partial f}{\partial x_1}$ indicates $Q_{GLZ\,12\,h}$ ($Y_3$) increases with CA:PEG-1500 ratio ($X_1$).

In Eqs. (6), the value range of $\frac{\partial f}{\partial x_1}$ is an interval from 0.071 to -0.016, and the value range of $\frac{\partial f}{\partial x_2}$ is an interval from 0.055 to -0.011. The change of partial derivative indicates $RSQ_{GLZ\,zero}$ ($Y_4$) increases with CA:PEG-1500 ratio ($X_1$) and weight gain ($X_2$).

Fig. 3c and Fig. 3d illustrate the quadratic relationship between the CA: PEG-1500 ratio and weight gain. The increase in CA: PEG-1500 ratio from 4 to 6 and weight gain from 2.5 to 4.5 results in fall in the graph of $Q_{GLZ\,12\,h}$ and rise in the graph of $RSQ_{GLZ\,zero}$. The graphical analysis is coincident with the mathematical analysis.

Therefore, the similarity of release characteristics of CA: PEG-1500 ratio and weight gain indicates the release of MTF and GLZ are affected by the two factors synchronizely.

**Formulation optimization**
and $Y_3$ are cumulative release percentage and expected to be maximized, while $Y_2$ and $Y_4$ are $R^2$ of drug release data fitted to zero-order equation and expected to be close 1. Based on this standard, the optimized regions are represented in red color in Fig.3. The overlapping region shows the optimal formulation in response to every factor. The relationship between experimental values and predicted ones are in agreement (Table 5). The cumulative release profile of the optimized formulation is illustrated in Fig.4. The $f_2$ value of the release of MTF and GLZ is 70, which indicates the two drugs release synchronously.

**In vivo study in beagle dogs**

The main pharmaceutical parameters, such as $C_{\text{max}}$, $T_{\text{max}}$, $\text{AUC}_{(0-24\ h)}$ and $\text{AUC}_{(0-\infty)}$ are listed in Table 6. Fig. 5a-b shows the pharmacokinetics profiles in beagle dogs of the optimized formulation. In comparison with conventional tablets, drug plasma concentration of optimized formulation rises with relatively low peak. The relative bioavailability of optimized formulation is 99.2% and 99.3% for MTF and GLZ, respectively. The 90% confidence interval of the AUC ($0-\infty$) of optimized formulation is 84.9-113.8% for MTF and 83.2-112.3% for GLZ. Moreover, by analysis of DAS 2.0 software and Wagner-Nelson method, it displays acceptable correlation parameter ($R = 0.9699$ for MTF and 0.9595 for GLZ) which implies *in vitro* drug release is in agreement with *in vitro* absorption.

**Conclusion**

In this study, compound EOP tablet of MTF and GLZ is designed to take advantage of the combination of two drugs and achieve prolonged steady blood drug level. In this EOP system, MTF is not only an active ingredient, but also acts as an osmotic agent to generate
sufficient osmotic pressure to facilitate the release of GLZ. Among all the factors in relation
with the release rate of the drugs, pore-forming agent ratio and membrane coating thickness
play an important role. Moreover, the formulation of EOP tablet is optimized by a
face-centered central composite design (FCCD) for better controlled release profile. Then the
optimal formulation is further validated both by in vitro and in vivo study, which shows
zero-order release profile in vitro and displays prolonged blood drug concentration-time
profile in vivo. At the same time, in vitro and in vivo correlation for MTF and GLZ of the
EOP tablet is desirable. Overall, a highly water-soluble drug MTF and poorly water-soluble
drug GLZ are delivered in sustained and synchronized manner in vitro and in vivo.

Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content
and writing of this paper.

Reference
1. Marije VD, Bannink EMN, Pareren YK, Van, et al. Risk factors for diabetes mellitus type 2 and
metabolic syndrome are comparable for previously growth hormone-treated young adults born small
for gestational age (sga) and untreated short SGA controls. J Clin Endocrinol Metab.


Table 1 Formulations used for the design of elementary osmotic pump tablets

Table 2 Variables in $3^2$ face-centred central composite design

Table 3 Matrix of $3^2$ face-centred central composite design

Table 4 Regression Equations and Statistical Analysis

Table 5 Optimal factors and the predicted values as well as actual results of the optimized formulation

Table 6 Pharmacokinetics parameters of MTF and GLZ in beagle dogs ($n = 6$)
<table>
<thead>
<tr>
<th>Formulations</th>
<th>Core tablet</th>
<th>Coating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTF (mg)</td>
<td>GLZ (mg)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5</td>
</tr>
<tr>
<td>F&lt;sub&gt;initial&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F01, F02, F03</td>
<td>500</td>
<td>5</td>
</tr>
<tr>
<td>F04, F05, F06</td>
<td>500</td>
<td>5</td>
</tr>
<tr>
<td>F07, F08, F09</td>
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<td>5</td>
</tr>
<tr>
<td>F10, F11, F12</td>
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<td>5</td>
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<tr>
<td>F13, F14, F15</td>
<td>500</td>
<td>5</td>
</tr>
<tr>
<td>F16, F17, F18</td>
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### Table 2

<table>
<thead>
<tr>
<th>Independent variable, factor</th>
<th>Levels used</th>
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<tr>
<td></td>
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<tr>
<td>$X_1 = \text{CA:PEG-1500 ratio}$</td>
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<tr>
<td>$X_2 = \text{Weigh gain (%)}$</td>
<td>2.5</td>
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Table 3

<table>
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<tr>
<th>Formulation batches</th>
<th>Coded factors</th>
<th>Actual values of variable</th>
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<tr>
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<td>$X_1$</td>
<td>$X_2$</td>
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<td>Factorial points</td>
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<tr>
<td>B₁</td>
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<td>1</td>
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<tr>
<td>B₂</td>
<td>−1</td>
<td>−1</td>
</tr>
<tr>
<td>B₃</td>
<td>−1</td>
<td>1</td>
</tr>
<tr>
<td>B₄</td>
<td>1</td>
<td>−1</td>
</tr>
<tr>
<td>Center points</td>
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<td></td>
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<tr>
<td>B₅</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B₆</td>
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<tr>
<td>B₇</td>
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<tr>
<td>B₉</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Axial points</td>
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<td></td>
</tr>
<tr>
<td>B₁₀</td>
<td>−1 (−α)</td>
<td>0</td>
</tr>
<tr>
<td>B₁₁</td>
<td>0</td>
<td>−1 (−α)</td>
</tr>
<tr>
<td>B₁₂</td>
<td>1 (+α)</td>
<td>0</td>
</tr>
<tr>
<td>B₁₃</td>
<td>0</td>
<td>1 (+α)</td>
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Table 4

<table>
<thead>
<tr>
<th>Term</th>
<th>Model fitting</th>
<th>P-value</th>
<th>Predicted (R^2)</th>
<th>Adjusted (R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Y_1)</td>
<td>(Y_1 = -68.16516 + 53.57930 \times X_1 + 34.74313 \times X_2 - 4.59750 \times X_1 \times X_2 - 4.64414 \times X_1^2 - 2.62414 \times X_2^2)</td>
<td>&lt; 0.0001</td>
<td>0.851</td>
<td>0.958</td>
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<tr>
<td>(Y_2)</td>
<td>(Y_2 = +0.24732 + 0.14913 \times X_1 + 0.15947 \times X_2 - 0.017050 \times X_1 \times X_2 - 6.66897 \times 003 \times X_1^2 - 8.11897 \times 003 \times X_2^2)</td>
<td>&lt; 0.0001</td>
<td>0.874</td>
<td>0.969</td>
</tr>
<tr>
<td>(Y_3)</td>
<td>(Y_3 = +53.82375 + 18.96708 \times X_1 + 8.47917 \times X_2 - 2.35250 \times X_1 \times X_2 - 1.58000 \times X_1^2 - 0.19000 \times X_2^2)</td>
<td>&lt; 0.0001</td>
<td>0.993</td>
<td>0.996</td>
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<tr>
<td>(Y_4)</td>
<td>(Y_4 = +0.041355 + 0.21522 \times X_1 + 0.16811 \times X_2 - 0.019575 \times X_1 \times X_2 - 0.011926 \times X_1^2 - 6.87586 \times 003 \times X_2^2)</td>
<td>&lt; 0.0001</td>
<td>0.955</td>
<td>0.979</td>
</tr>
</tbody>
</table>

\(Y_1\) (QMTF12 h): percentage of MTF released within 12 h; \(Y_2\) (RSQMTF zero): \(R^2\) of MTF release data fitted to zero-order equation;

\(Y_3\) (QGLZ12 h): percentage of GLZ released within 12 h; \(Y_4\) (RSQGLZ zero): \(R^2\) of GLZ release data fitted to zero-order equation.
### Table 5

<table>
<thead>
<tr>
<th>$X_1$</th>
<th>$X_2$ (%)</th>
<th>Response</th>
<th>Predicted value</th>
<th>Actual value</th>
<th>Bias (%)</th>
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<tr>
<td></td>
<td>Y_1 (%)</td>
<td></td>
<td>92.63</td>
<td>93.51</td>
<td>0.9500</td>
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<tr>
<td>5:1</td>
<td>Y_2</td>
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<td>0.9865</td>
<td>0.9860</td>
<td>−0.0506</td>
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<tr>
<td></td>
<td>Y_3 (%)</td>
<td></td>
<td>95.34</td>
<td>95.27</td>
<td>−0.0734</td>
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<tr>
<td></td>
<td>Y_4</td>
<td></td>
<td>0.9809</td>
<td>0.9829</td>
<td>0.2039</td>
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### Table 6

<table>
<thead>
<tr>
<th>Formulation</th>
<th>MTF</th>
<th>GLZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{\text{max}}$</td>
<td>$T_{\text{max}}$</td>
</tr>
<tr>
<td>Conventional tablet</td>
<td>12.28 ± 2.73</td>
<td>1.42 ± 0.38</td>
</tr>
<tr>
<td>EOP tablet</td>
<td>6.36 ± 1.95</td>
<td>4.08 ± 0.97</td>
</tr>
</tbody>
</table>
**Fig. 1** *In vitro* release profiles of the initial formulation of MTF and GLZ.

**Fig. 2a** *In vitro* release profiles of MTF with different core tablets
F01, F02 and F03 show the impact of NaHCO₃ on MTF release, while F04, F05, F06 and F07 show the effect of release retardant on MTF release.

**Fig. 2b** *In vitro* release profiles of GLZ with different core tablets
F01, F02 and F03 show the impact of NaHCO₃ on GLZ release, while F04, F05, F06 and F07 show the effect of release retardant on GLZ release.

**Fig. 2c** *In vitro* release profiles of MTF with different coating membrane
F10, F11, F12, F13, F14 and F15 show the impact of different pore-forming agents on MTF release, while F16, F17 and F18 show the effect of coating weight gain on MTF release.

**Fig. 2d** *In vitro* release profiles of GLZ with different coating membrane
F10, F11, F12, F13, F14 and F15 show the impact of different pore-forming agents on GLZ release, while F16, F17 and F18 show the effect of coating weight gain on GLZ release.

**Fig. 3** Response surface for (a) the release percent of MTF within 12 h ($Y_1$), (b) $R^2$ of MTF release data fitted to zero-order equation ($Y_2$), (c) the release percent of GLZ within 12 h ($Y_3$), and (d) $R^2$ of GLZ release data fitted to zero-order equation ($Y_4$) as function of CA:PEG-1500 ratio ($X_1$) and weigh gain ($X_2$).

**Fig. 4** *In vitro* release profiles of the optimized formulation with MTF and GLZ.

**Fig. 5** *In vivo* pharmacokinetics profiles of (a) MTF and (b) GLZ in beagle dogs from the conventional tablets and the EOP tablets ($n = 6$).

**Fig. 6** *In vivo-in vitro* correlation for MTF and GLZ of the EOP tablets.

Figure 1
Figure 2a
Figure 3
Figure 5b

[Graph showing concentration of GLZ in plasma vs. time for conventional and EOP tablets]

Figure 6

[Graph showing in vivo absorbed fraction vs. in vitro cumulative release for MTF and GLZ, with linear fits]