AAPS PharmSciTech, Vol. 16, No. 2, April 2015 (© 2014)
DOI: 10.1208/s12249-014-0229-1

Research Article

Bilayer Matrix Tablets for Prolonged Actions of Metformin Hydrochloride and Repaglinide

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Received 13 June 2014; accepted 18 September 2014; published online 16 October 2014

Abstract. A combination therapy of metformin hydrochloride (MH) and repaglinide (RG) achieves a perfect glycemic control; however, the combination formulation of immediate release must be taken several times a day, compromising the therapeutic benefits and causing inconveniences to the patients. Herein, a bilayer matrix tablet that aimed at continuously releasing both MH and RG over time was developed, in which the two drugs were formulated into two separated layers. The tablets were prepared by wet granulation method, and the optimized formulation was obtained by evaluating the factors that affected the drug release. The bilayer tablets simultaneously released the two drugs over 12 h; and a better *in vivo* performance with a steady plasma concentration, markedly lower C_{max}, prolonged T_{max}, and perfect absorption was obtained. Summarily, the bilayer matrix tablets sustained both MH and RG release over time, thereby prolonging the actions for diabetic therapy and producing better health outcomes.

KEY WORDS: bilayer tablets; metformin hydrochloride; pharmacokinetics; repaglinide; sustained release.

INTRODUCTION

Diabetes mellitus, which type 2 diabetes (T2D) takes up at least 90% of all cases, is one of the most common chronic diseases worldwide, producing great burden for the progressive aging of population and worsening of lifestyle (1). China has become a country with the greatest number of people with diabetes in the world, 92.4 million adults who accounts for 10% of the population suffering from the disease (2,3).

Metformin hydrochloride (MH) with perfect water solubility is an oral hypoglycemic drug for the treatment of T2D, decreasing the blood glucose levels by a mechanism of enhancing peripheral and hepatic sensitivity to insulin (4,5). Repaglinide (RG), another poorly water-soluble antidiabetic drug for T2D, lowers the glucose levels by stimulating insulin secretion from the pancreatic beta cells by closing ATP-dependent potassium channels and facilitating postprandial insulin response (6).

However, two defects, defective insulin sensitivity and defective insulin secretion, coexist in most patients with T2D, resulting in the fact that monotherapy with the oral antidiabetic drugs often fails to reduce the glucose levels (7). Thus, an ideal approach for T2D treatment should correct the two defects at the same time. Combination therapy acting by complementary mechanisms of action provides a perfect glycemic control for patients with T2D while reducing the side effects (8,9).

Based on the action mechanisms of MH and RG for T2D therapy, a synergistic effect for reducing the glucose levels can be achieved by a combination therapy of the two drugs. Therefore, MH-RG compound tablets (PrandiMet®, Novo Nordisk Inc.) were developed and approved by FDA in 2008. It is very popular with patients because the marked product can maintain the glycemic control over time. However, to keep the therapeutic concentration, the combination formulation of immediate release (IR) must be taken with high doses for several times each day because of the short half-life (less than 2 h) of the drugs (6,10). It not only compromises the therapeutic benefits and causes inconveniences to patient, but also leads to fluctuations of plasma concentration and induces unwanted side effects (11). Furthermore, a long-term administration of IR formulation may also generate poor adherence for treatment of chronic diseases, developing resistance to therapy and thus producing bad health outcomes (9). Therefore, the development of a prolonged action preparation for the combination formulation of MH and RG is urgent.

The matrix bilayer tablet with two separated release-layers is a biphasic delivery system that is purposed to release a drug at two different rates or simultaneously deliver two drugs. Its advantages include (12,13) formulating two

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incompatible drugs into a system, simultaneously delivering two drugs with desired release profiles, increasing the drug efficacy by synergistic effect, reducing the dosing unit burden, improving patient compliance, low cost, and ease of manufacture.

To overcome the drawbacks of combination IR formulation of MH and RG, we previously reported a sandwiched osmotic pump system for the two drugs, which both of them were released at a zero-order rate (14); however, the preparation process was too complicated to scale up. Thus, a new dosage form that is prepared by a more simple process and continuously releases the two drugs over time is required. Accordingly, the bilayer matrix tablets meet the requirements because of the benefits as described above. Therefore, the present study was to (1) design the bilayer matrix tablets that formulated MH and RG into the isolated layers and simultaneously delivered the two drugs with a sustained release profile, (2) optimize the formulations for the tablets, (3) evaluate the pharmacokinetics of the tablets in dogs, and (4) assess the *in vitro–in vivo* correlations.

MATERIALS AND METHODS

Materials

MH was from Jiangsu Hansitong Pharmaceutical Co., Ltd. (Changsu, China). RG was purchased from Zhejiang Haixiang Pharmaceutical Co., Ltd. (Taizhou, China). Hydroxypropylmethycellulose (HPMC K4M, K100M) and ethylcellulose (Ethocel®, EC) were obtained from Colorcon Co., Ltd. (Shanghai, China). Polyoxyethylene (PEO) used in the form of Polymox N60k (MW 2 million) was from Dow Chemical Co., Ltd. (Shanghai, China). PVP K30 was gifted from ISP Tech., Ltd. (Shanghai, China). Lactose was obtained from Meggle Pharmaceutical Co., Ltd. (Wasserburg, Germany). Magnesium stearate was obtained from Anhui Shanhe Adjuvant Co., Ltd. (Huannan, China). Methanol and other reagents were of chromatographic grade.

Preparation of Sustained Release Bilayer Tablets

To enhance the drug solubility, a solid dispersion of RG/PVP K30 (Table I) with the ratio of 1:10 (*w*/*w*) was prepared before the granulation process (15).

The granules of drug layer were prepared by a wet granulation method. Before the granulation process, the drug and other raw materials were dried at 60°C for 2 h and then passed through an 18-mesh screen (14).

MH Layer

MH and other inactive ingredients were mixed by an equal incremental method. Then the granules of MH layer were prepared using 5% solution of HPMC as binder. The dry granules were pressed through a 20-mesh screen and then mixed with 1% (*w*/*w*) magnesium stearate.

RG Layer

The preparation procedure of granules of RG layer was similar to that of MH layer. The solid dispersion of RG/PVP K30 was mixed with other adjuvant and then granulated with 5% solution of PVP K30. After being sieved through a 20-mesh screen, the dry granules were mixed with 1% (*w*/*w*) magnesium stearate.

The bilayer tablets were prepared using a single-punch tablet machine (TDP, Shanghai Tianxiang & Chentai Pharmaceutical Machinery Co. Ltd., Shanghai, China) by a double-compressing procedure. A fixed dose of the granules of drug layer was in sequence filled into the die cavity and then followed by a compression for each time. The batch size was 100 tablets for each formulation. The compressed pressure varied from 3 to 11 kg/cm^2.

In Vitro Drug Release

The drug release from the bilayer tablets was performed with Chinese Pharmacopeia type III apparatus (RCZ-8A, Tiandatianfa Tech., Ltd., Tianjin, China). Briefly, the tablets were placed into the cups with 100 mL of release medium and kept at 37±0.5°C with paddle rotation speed of 100 rpm. At the time points of 1, 2, 4, 6, 8, 12, and 24 h, 1 mL of release medium was withdrawn with a syringe filter (0.45 μm) and replaced with an equal volume of fresh medium. The determination of MH in samples was carried out using an UV spectrophotometer (UV-2401PC, Shimadzu Co., Ltd., Jiangsu, China) at 233 nm while RG analysis was performed by HPLC method described below.

Mechanisms of Drug Release

To obtain insight into the mechanism of drug release, the *in vitro* release data of optimized formulation was fitted to Peppas model (16):

$$\frac{M_t}{M_f} = k \bullet t^n \tag{1}$$

Where M_t and M_f are the percentages of drug release at time t and infinite time, respectively; k is a constant incorporating structural and geometric characteristics of the drug dosage form; and n is the release exponent, indicative of the mechanism of drug release.

Based on the values of release exponent, the drug release mechanism for a cylinder corresponded to Fickian release for $n<0.45$, non-Fickian release (anomalous transport) for $0.45<n<0.89$, case II release for $n=0.89$, and super case II for $n>0.89$ (17).

Pharmacokinetics in Dogs

All the animal experiments were conducted using protocols approved by the University Ethics Committee of Care and Use of Laboratory Animals. The animals were housed and handled according to the University Unit for Laboratory Animal Medicine guidelines.

A randomized, two-period crossover design was performed to study the pharmacokinetics of the optimized sustained release bilayer tablets (SR tablets, Table II) and commercially available immediate release tablets (IR tablets) in beagle dogs. Six male dogs with a body weight of 10–12 kg

Table I. Formulation Composition of Sustained Release Bilayer Tablets of MH and RG (All Qualities Were Given in Gram)

	MH layer									RG layer						
F	MH	HPMC K100M	Eudragit RS	ATO 888	EC 45p	CMC-Na (H)	CMC-Na (L)	HPMC E5	PVP K30	MCC	RG SD	EC 45p	Polyox N60K	HPMC K4M	ATO 888	MCC	Lactose
F1	250	25	–	–	–	25	–	QS	–	60	11	–	–	–	250	40	40
F2	250	–	25	–	–	25	–	QS	–	60	11	–	–	–	250	40	40
F3	250	–	–	25	–	25	–	QS	–	60	11	–	–	–	250	40	40
F4	250	–	–	–	25	25	–	QS	–	60	11	–	–	–	250	40	40
F5	250	25	–	–	–	25	–	QS	–	60	11	–	–	–	250	40	40
F6	250	25	–	–	–	–	25	QS	–	60	11	–	–	–	250	40	40
F7	250	25	–	–	–	25	–	–	QS	60	11	–	–	–	250	40	40
F8	250	25	–	–	–	25	–	QS	–	60	11	25	–	–	–	40	40
F9	*250*	*25*	–	–	–	*25*	–	*QS*	–	*60*	*11*	–	*25*	–	–	*40*	*40*
F10	250	25	–	–	–	25	–	QS	–	60	11	–	–	25	–	40	40
F11	250	25	–	–	–	25	–	QS	–	60	11	–	–	–	25	40	40
F12	250	25	–	–	–	25	–	QS	–	60	11	–	–	10	–	40	40
F13	250	25	–	–	–	25	–	QS	–	60	11	–	–	15	–	40	40
F14	250	25	–	–	–	25	–	QS	–	60	11	–	–	20	–	40	40
F15	250	25	–	–	–	25	–	QS	–	60	11	–	–	25	–	80	–

MH metformin hydrochloride, *RG* repaglinide, *F* formulation, *SD* solid dispersion, *HPMC* hydroxypropylmethycellulose, *EC* Ethocel®, *QS* quantity sufficient, *CMC-Na* sodium carboxyl methyl cellulose, *H* high viscosity, *L* low viscosity, *PVP K30* polyvinylpyrrolidone K30, *MCC* microcry stalline cellulose, *ATO 888* compritol 888 ATO

were randomly divided into two groups. After being fasted for 12 h prior to administration, the dogs were administered with IR and SR tablets (MH 250 mg and RG 1 mg, respectively). Four hours after administration, the dogs were given normal dog food with free access to water during the study. A washout period of 2 weeks between two consecutive administrations was allowed to eliminate the effect of the prior doses before the next drug administration.

Blood samples (3 mL) were collected from the upper limb at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 h for IR tablets, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, and 36 h for SR tablets after administration. All the blood samples were immediately transferred to heparinized centrifuge tubes and then centrifuged at 4, 000 rpm for 10 min. The supernatant plasmas were stored at −70°C until further analysis. The drug concentration in plasma was assayed by high performance liquid chromatography-electrospray tandem mass spectrometry (HPLC-MS/MS) method described below.

Sample Preparation and Analytical Methods

MH content in release samples was determined by UV at 233 nm, while RG measurement was performed on a Shimadzu HPLC 2010 system (Shimadzu, Japan).

RG was separated at 30°C using a ODS column (4.6 mm× 150 mm; Diamonsil, China) and detected at 287 nm. The mobile phase was a mixture of 0.005 mol/L ammonium acetate and methanol (20:80, *v*/*v*) pumped at a rate of 1.0 mL/min.

To determine the drug concentration in plasma, 200 μL of plasma sample and 20 μL of internal standard (200 ng/mL nateglinide in methanol) were transferred to a plastic tube and mixed by vortex. After being further added with protein precipitant (600 μL of acetonitrile), the mixture was vortexed for 60 s and then centrifuged at 12, 000 rpm for 10 min. Ten microliters of the supernatant was injected into the chromatographic system.

The HPLC-MS/MS system consisted of a LC-20AD pump, a CBM-20 communication bus module, a SIL-20AC auto-sampler, a CTO-20A column oven (Shimadzu, Japan), and a TSQ quantum access tandem mass spectrometer (Thermo Scientic, USA), equipped with an electrospray ionization

Table II. Optimized Formulation for Sustained Release Bilayer Tablets of MH and RG

MH layer (mg)	
MH	250
HPMC K100M	25
MCC101	60
CMC-Na (H)	25
Magnesium stearate	6
5% HPMC E5	QS
RG layer (mg)	
RG solid dispersion	11
HPMC K4M	25
MCC101	40
Lactose	40
Magnesium stearate	1
5% PVP K30	QS

MH metformin hydrochloride, *RG* repaglinide, *HPMC* hydroxypropylmethycellulose, *QS* quantity sufficient, *CMC-Na* sodium carboxyl methyl cellulose, *H* high viscosity, *PVP K30* polyvinylpyrrolidone K30, *MCC* microcry stalline cellulose

(ESI) ion source. The chromatographic separation was carried out at 40°C using a Shimadzu VP-ODS C_{18} column (4 μm, 2.0 mm×150 ;mm, Shimadzu) at a flow rate of 0.3 mL/min. The mobile phase was composed of 0.005 mol/L ammonium acetate aqueous solution (A) and acetonitrile (B), using a gradient according to the following profile: 0–1 min, 45% B; 1–3.5 min, 10% B; and 3.5–5.0 min, 45% B.

The conditions for mass spectrum was similar to a previous report (15), with following modifications: ion source, ESI source; detection mode, poison detection; atomization voltage, 3.5 kV; heating capillary temperature, 350°C; scabbard gas, N_2; pressure, 40 Arb; assistance gas, N_2; pressure, 10 Arb; crash gas, Ar; pressure, 1.5 Torr; CID voltage, MH, 21 eV; RG, 30 eV; enalaprilat,17 eV; scan mode, SRM; ionic reaction of quantitative analysis, MH, m/z 130.2→m/z 71.4; RG, m/z 453.3→m/z 230.1; enalaprilat, m/z 349.4→m/z 206.1. The correlation coefficients of the calibration curves were more than 0.998. Absolute recovery of low, medium, and high QC levels were ranged from 96–108%; and the CV of inter-day and intra-day assay was less than 15%.

Data Analysis and Statistics

The pharmacokinetic parameters were obtained by a statistical moment theory. $P<0.05$ was considered as a significant difference. The relative bioavailability (F) of the test formulation was calculated by the ratio of the AUC values of test formulation and reference formulation.

RESULTS AND DISCUSSION

Preparation of Sustained Release Bilayer Tablets

In theory, a synergistic effect for T2D treatment can be achieved by the combination therapy of MH and RG; however, MH is a drug with high water solubility while RG is a BCS class II drug with poor water solubility, and the dissolution rate is its absorption limiting-step in the gastrointestinal tract. It thus results in a bad compatibility for dosage form design.

To improve the solubility and absorption of RG, the solid dispersion using PVP K30 as carrier was prepared. Due to the presence of amorphous form, the solubility and bioavailability were enhanced greatly (15). Thus, the active ingredient of solid dispersion of RG/PVP K30 was formulated into the tablets.

In order to simultaneously deliver the two drugs with a sustained release profile, the bilayer matrix tablets prepared by a double compressing procedure were developed by incorporating them into the separated layers (Fig. 1). Herein, the formulations for tablets, which their compositions are shown in Table I, were optimized by studying the factors that affected the *in vitro* drug release.

Factors Affecting MH Release

The influence of matrix material, release modifier, binder, and compression pressure on the MH release in water is shown in Fig. 2.

The MH release was significantly affected by the types of sustained release material (Fig. 2a, Table I F1–F4). The release of MH was 54, 80, 55, and 73% at 4 h and 77, 97, 73, and 94% at 8 h for the tablets with HPMC K100M, EC 45cp, ATO 888 and Eudragit RS as matrix, respectively. The hydrophilic polymers retarded the drug release more significantly than that of hydrophobic polymers. It was ascribed to the difference in mechanism of drug release (18–20). In detail, the former (hydrophilic polymers) released the water-soluble drug in a diffusion-controlled manner while the latter (hydrophobic polymers) released the drug via a mechanism of erosion and diffusion. It was also reported that the matrix of hydrophobic polymer after being exposed to water would disintegrate within a short time, thus allowing a faster drug release (21). Both ATO 888 and HPMC K100M sustained the drug release up to 12 h; however, ATO 888 with lower melting point would lead to stick punch, affecting scaling-up production (22). Thus, HPMC K100M was used as matrix material in MH layer.

To prevent the effects of burst release and doses-pumping of a freely water-soluble drug, a release modifier like ionic polymer should be incorporated into the formulation, aiming at enhancing the robustness and resistance against stronger stresses of gel layer of HPMC matrices (23). Herein, CMC-Na was added into the formulation (Table I F5, F6). It was observed that the higher the viscosity of the polymer was, the slower the drug release was (Fig. 2b). Importantly, the drug release profile from the formulation with a combination of HPMC K100M and CMC-Na was similar to the formulation with single HPMC K100M, indicating that the release rate was not changed and a stable drug release in gastrointestinal tract without any drug burst caused by disintegration was obtained because the two formulations had similar diffusion exponent (24).

The types of binder also produced influence on MH release, which the formulations with HPMC E5 as binder exhibited a slower drug release (Fig. 2c, Table I F5, F7). It was since that HPMC E5 possessed higher viscosity and was dissolved in water with a slower rate, hindering the erosion of the polymer matrix.

To further study the compression pressure effect on MH release, the tablets of optimized formulation with hardness of around 3, 6, and 9 kg were prepared (Table II). It was observed that the release of MH was slightly decreased with an increase in the compression pressure (Fig. 2d), owing to the decreased rate of water uptake and hydration of polymer.

Factors Affecting RG Release

The effects of matrix material, amount of HPMC K4M, filler, particle size of solid dispersion, and compression pressure on the release profiles of RG in water are depicted in Fig. 3.

Among the four types of matrix material (Table I F8–F11), HPMC K4M and ATO 888 sustained the release of RG for a longer time, continuously delivering the drug for 24 h (Fig. 3a). For a poorly water-soluble drug, its release from a matrix was of both swelling and erosion-dependence. EC 45cp was an insoluble polymer whose skeleton of matrix would be collapsed within 1 h after being immersed in aqueous condition, thus resulting in a fast drug release (21). In contrast, the drug release from a matrix formed with hydrophilic polymer like HPMC K4M or Polyox N60K was controlled by the synchronization of swelling and erosion of polymer matrix,

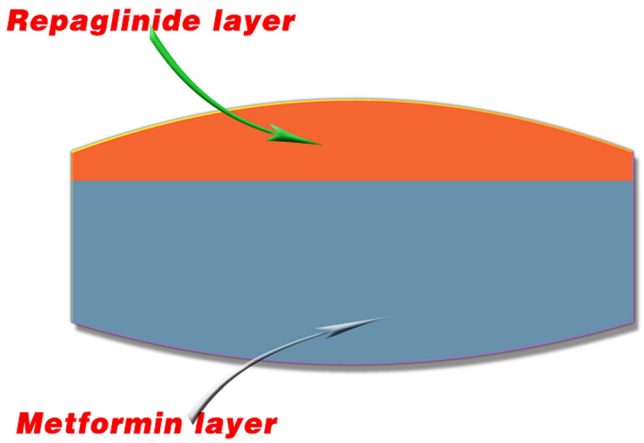

Fig. 1. Schematic illustration of bilayer matrix tablet for simultaneously sustaining release of MH and RG

and thus, a lower rate of drug release always occurred (25). The drug release from the lipid matrix system formed with ATO 888 only relied on the erosion of the matrix, leading to further slowing down of the drug release as compared to that of hydrophilic matrix. However, due to the stick punch caused by ATO 888, HPMC K4M was chosen as a sustained release material in RG layer.

As expected, increasing the amount of sustained release material (HPMC K4M) resulted in increasing the lag time and reducing the rate of RG release (Fig. 3b, Table I F10, F12–F14), ascribing to an increase in gel thickness and diffusion path length. Decreasing the particle size of solid dispersion containing RG led to an increase in drug release because a larger surface area for drug release was available (Fig. 3c). Similar to the MH release, the RG release was declined significantly as the compression pressure was increased up to 9 kg/cm^2 (Fig. 3d). It was because an increase of compression force would lead to a greater apparent density and lower matrix porosity.

Interestingly, the hydrophobic nature of filler significantly affected the RG release (Fig. 3e, Table I F10, F14, F15), evident by the fact that less than 60% of RG was released from the formulation with MCC as filler while the formulation filled with lactose achieved more than 80% of release at 12 h.

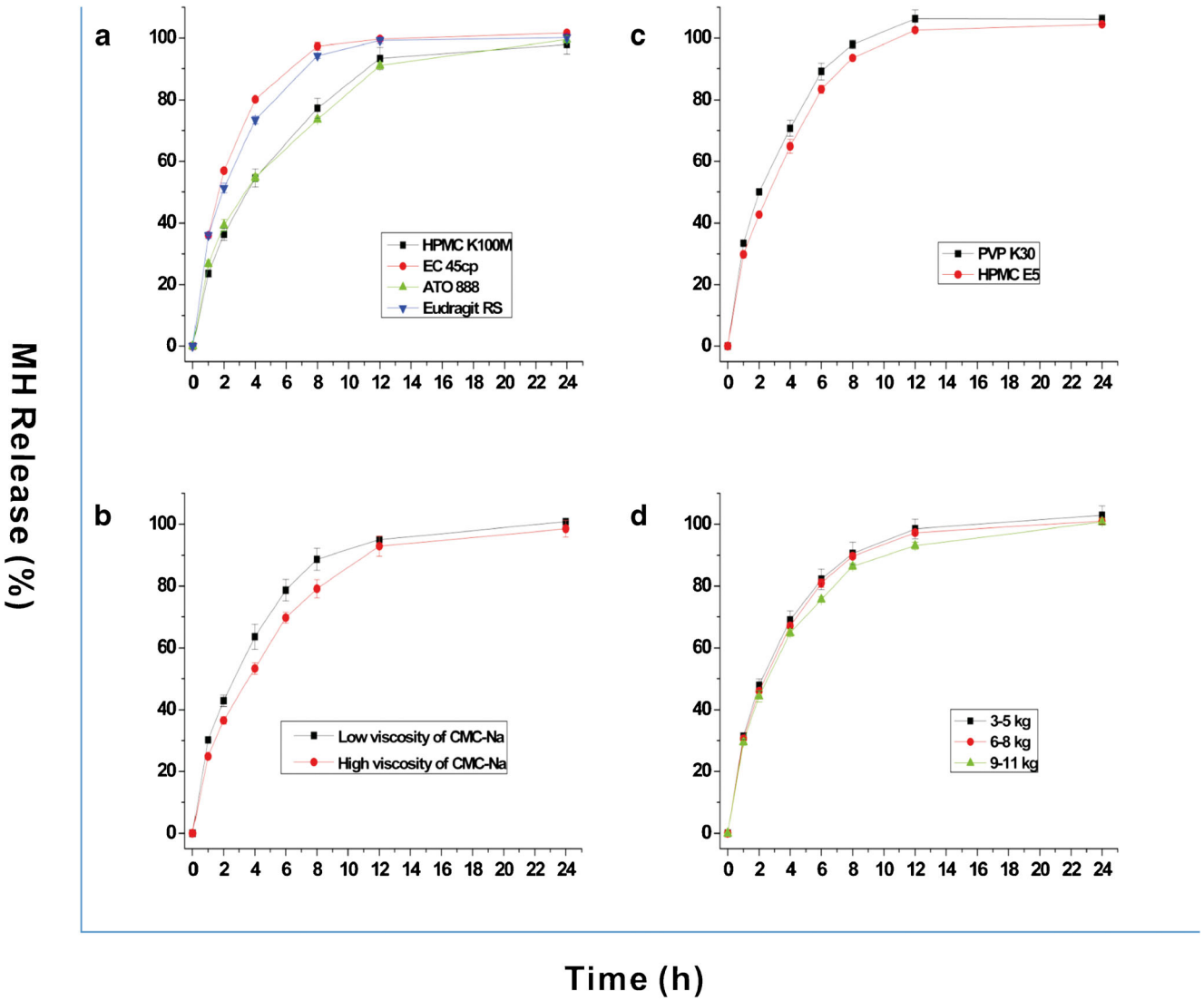

Fig. 2. Influence of **a** matrix material, **b** viscosity of CMC-Na, **c** binder, and **d** compression pressure on the release profiles of MH from sustained release bilayer tablets in water

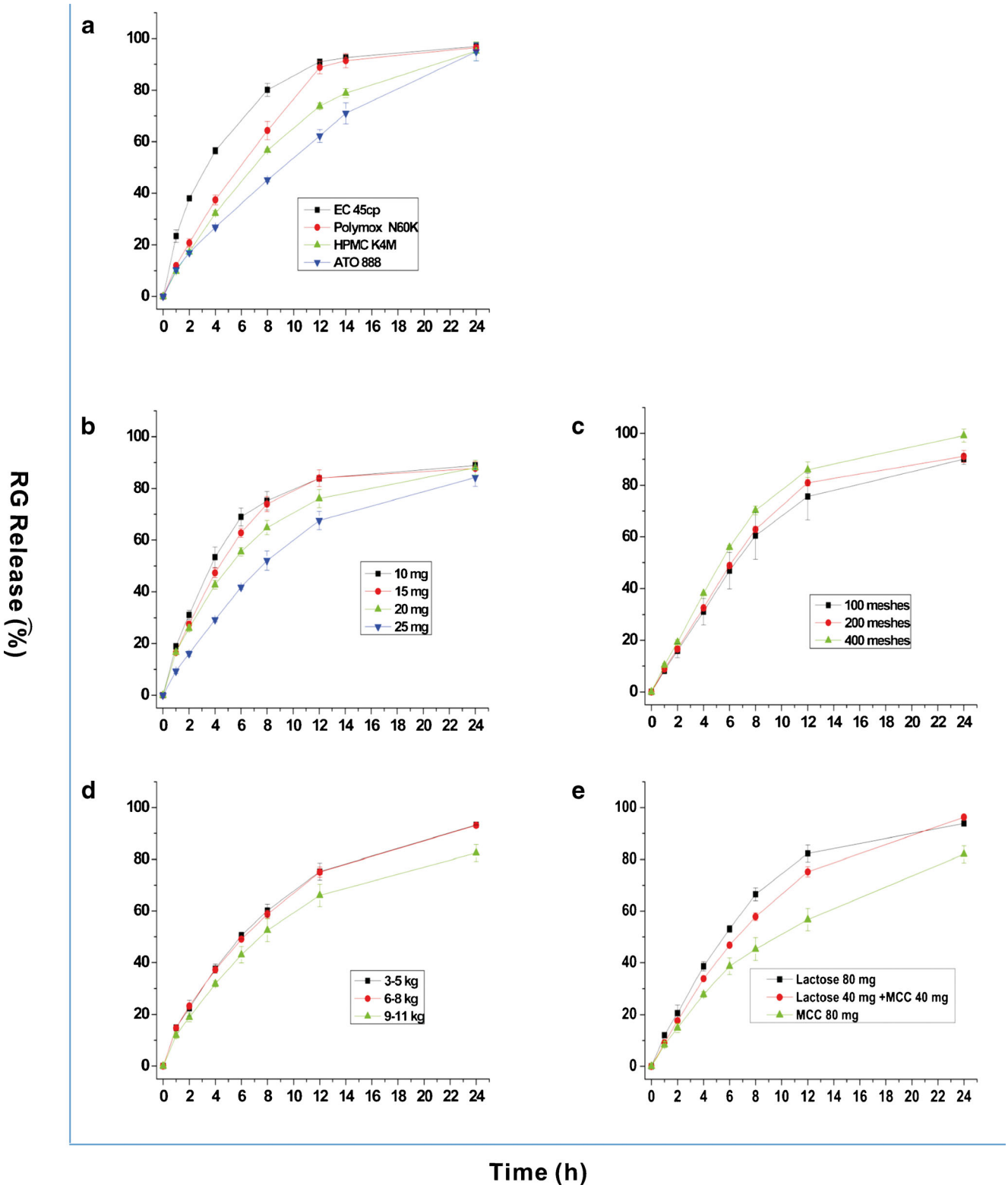

Fig. 3. Influence of **a** matrix material, **b** amount of HPMC K4M, **c** particle size of RG/PVP K30 solid dispersion, **d** filler, and **e** compression pressure on the release profiles of RG from sustained release bilayer tablets in water

It indicated that the drug release was reduced with an increase in the hydrophobicity of filler. Generally, the increased hydrophobicity of filler refrained the release fluid penetrating into the matrix system, decreased the osmotic pressure in matrix, and thereby retarded both of hydration and swelling of polymer (26), which the phenomenon was more profound for a hydrophilic matrix loading an insoluble drug (27).

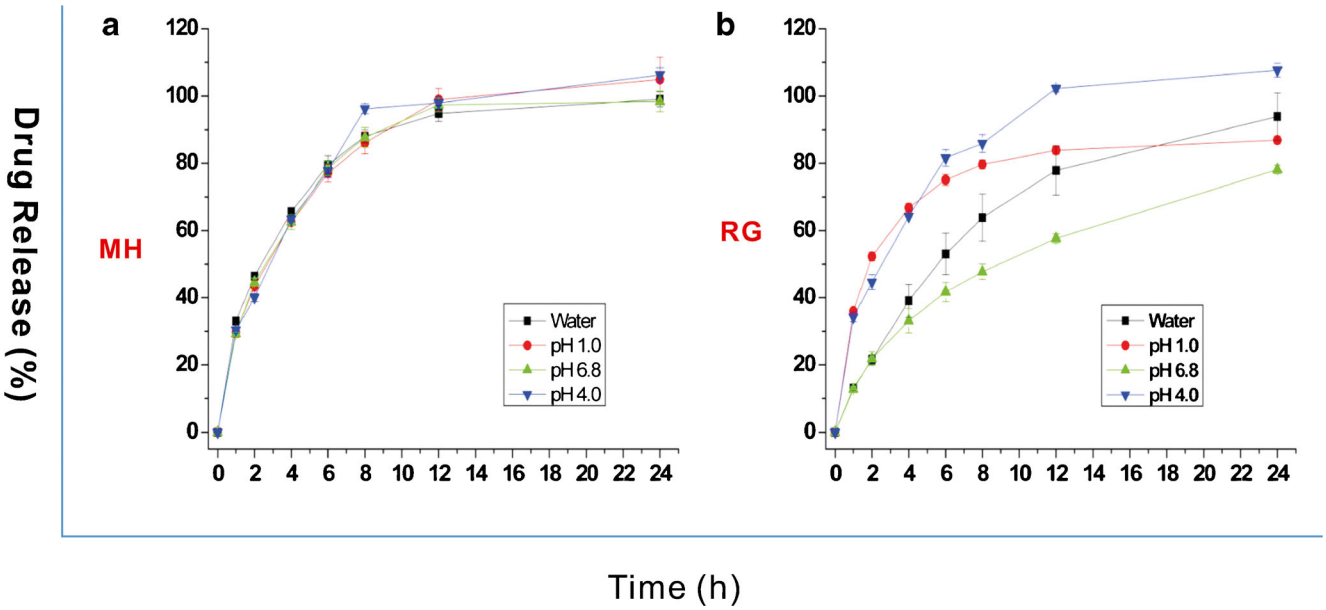

Fig. 4. Influence of medium pH on *in vitro* release of **a** MH and **b** RG from optimized formulation of sustained release bilayer tablets

Effect of Medium pH on MH and RG Release

The optimized formulation and drug release profiles in different mediums are shown in Table II and Fig. 4. There was no influence of medium pH on MH release, indicating that the *in vivo* drug release would not be affected by the pH change of gastrointestinal fluid. However, the *in vitro* release of RG was greatly influenced by the medium pH. RG was a zwitterionic drug with pH-dependent solubility, and its solubility at pH between 1 and 4 was significantly higher than that of the pH ranging from 5 to 8 (28,29), thus exhibiting a faster drug release in the medium of pH 1 and 4. Surprisingly, the rate of RG release in water was markedly less than that of in pH 6.8 medium, owing to the ions in release medium hampered the hydration of hydrophilic matrix and thus formed a weakening of gel layer (25). It was also observed that the RG release after 8 h in pH 4 medium was higher than that in pH 1, though the solubility in the latter was significantly greater than in the former. To some extent, the presence of drug particles in the HPMC gel layer would make the matrix more erodible, which certainly led to a faster release (27,30). Due to the effect of medium pH on RG release, a further study should be carried out to evaluate the *in vivo* performance.

Mechanisms of Drug Release

The release kinetics analyses of the MH and RG from the optimized formulation of sustained release bilayer tablets are presented in Table III. The release data of the two drugs in four different mediums fit well with the Peppas model, evident by the values of correlation coefficient (R) that were more than 0.99. The values of exponent from MH release were within the range of 0.45–0.89, indicating a non-Fickian release mechanism that the drug release was governed by a combination of diffusion and polymer erosion.

The RG release exponent obtained from water and medium of pH 6.8 and pH 4.0 was covered by the range of 0.45–0.89, therefore suggesting that the RG release also follows a non-Fickian release mechanism. However, the RG exponent from pH 1 medium was less than 0.45; thus it indicated that its release was controlled by a diffusion-dependent manner. The change in release mechanism was ascribed to a higher solubility of RG in acid conditions, which the solubility at pH 1 medium was up to 1.8 mg/mL while it was less than 0.081 mg/mL in other mediums (28,29).

Table III. Korsmeyer–Peppas Model Fitting of Release Data of Sustained Release Bilayer Tablets of MH and RG. (n=3)

	MH			RG		
	Correlation coefficient (R)	Diffusion exponent (n)	Order release	Correlation coefficient (R)	Diffusion exponent (n)	Order release
Water	0.9992	0.48	Anomalous transport	0.9993	0.78	Anomalous transport
pH 1	0.9992	0.52	Anomalous transport	0.9910	0.41	Fickian diffusion
pH 6.8	0.9985	0.53	Anomalous transport	0.9972	0.65	Anomalous transport
pH 4	0.9955	0.56	Anomalous transport	0.9959	0.49	Anomalous transport

MH metformin hydrochloride, *RG* repaglinide

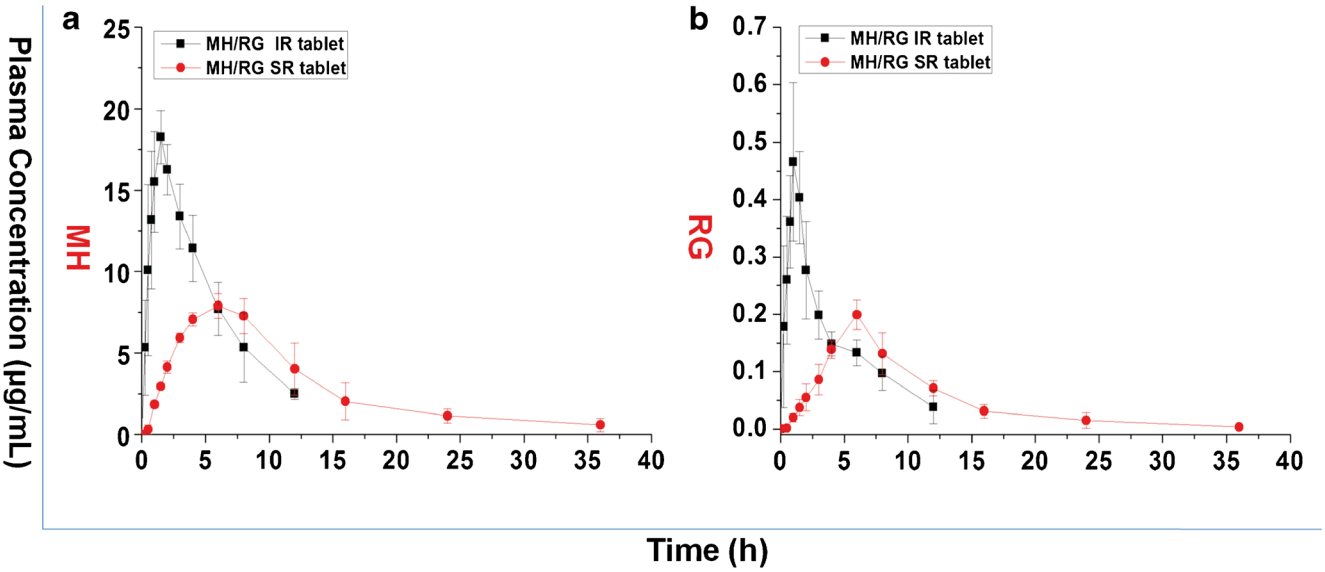

Fig. 5. Plasma **a** MH and **b** RG concentration as a function of time after single oral dose of 1000 mg/kg (MH) and 4 mg/kg (RG) of immediate release tablets (IR tablets) and sustained release bilayer tablets (SR tablets) (n=6)

Pharmacokinetics in Dogs

The plasma concentration-time profiles of MH and RG after administration of immediate release tablets (IR tablets) and sustained release bilayer tablets (SR tablets) in beagle dogs are shown in Fig. 5.

The values of pharmacokinetic parameters of the two drugs are summarized in Table IV. The values of C_{max} and T_{max} of IR tablets were about 18.25 μg/mL and 1.5 h for MH and 0.47 μg/mL and 1 h for RG, with the drug concentration in plasma rapidly rising with fast elimination. In contrast, the C_{max} and T_{max} from SR tablets were 7.90 μg/mL and 6 h for MH and 0.20 μg/mL and 6 h for RG. The C_{max} was reduced by 57% for both MH and RG while the time for T_{max} was prolonged by 3 times for MH and 5 times for RG than IR tablets, which was further confirmed by the prolonged MRT of SR tablets. Compared to the IR tablets, the values of F (%) were 103% for MH and 102% for RG, indicating a bioequivalence. Thus, the two drugs were simultaneously delivered with a prolonged action using the sustained release bilayer tablets.

It should be noted that the T_{max} and MRT of MH from SR tablets were almost as great as that of RG, suggesting that the *in vivo* release of the two drugs was synchronous and thus an enhanced synthetic effect for T2D treatment would be achieved. The two drugs were different in solubility and elimination half-life; however, a very similar *in vivo* performance was obtained by loading them into our designed SR tablets, indicating that the formulation was strong in modifying the *in vivo* release.

The RG release was affected by the medium pH because of its pH-dependent solubility, but a perfect *in vivo* performance was still obtained, indicating that the present formulation could overcome the change of gastrointestinal pH. It should be ascribed to the incorporation of RG solid dispersion into the formulation. RG belonged to a BCS II drug with low aqueous solubility and high permeability, thus, its absorption enhancement was obtained by increasing the dissolution rate. A previous report indicated that its bioavailability was increased up to 2.2 folds by solid dispersion; moreover, the absorption predominantly occurred in the upper gastrointestinal tract, especially in the segment of the duodenum and jejunum (15). On the other side, it was observed that the values of MH AUC obtained from the IR and SR tablets were equal, whereas the absorption of MH was site-dependent and the drug was mainly absorbed in the proximal small intestine (31,32). Thus, it was speculated that a complete release of both MH and RG was achieved in the upper gastrointestinal tract, which the SR tablets would not be transferred to the large intestine, enabling the drug to be well absorbed.

In Vivo–In Vitro Correlation

The absorption was limited by the *in vitro* drug release from a sustained release formulation, thus allowing an

Table IV. Pharmacokinetic Parameters of MH and RG After Administration of Immediate Release Tablets (IR Tablets) and Sustained Release Bilayer Tablets (SR Tablets) in Beagle Dogs. (n=6)

Formulation		C_{max} (μg/mL)	T_{max} (h)	$T_{1/2}$ (h)	MRT (h)	$AUC_{(0\text{-}t)}$ (μg/mL h)	F (%)
IR tablets	MH	18.25±1.01	1.50±0.41	3.44±0.80	5.20±2.17	101.20±8.64	–
	RG	0.47±0.12	1.00±0.29	3.44±1.57	6.72±1.76	2.26±0.34	–
SR tablets	MH	7.90±0.59	6.00±1.00	3.91±0.13	11.89±0.47	102.98±10.80	101.70±2.10
	RG	0.20±0.08	6.00±0.25	4.07±1.51	10.05±2.15	1.79±0.198	97.60±0.80

MH metformin hydrochloride, *RG* repaglinide, *F* relative bioavailability, *h* hour, $AUC_{0\text{-}t}$ area under the plasma concentration–time curve up to the last time point, C_{max} maximum plasma concentration, T_{max} time of maximum concentration, $T_{1/2}$ terminal half-life, *MRT* mean retention time

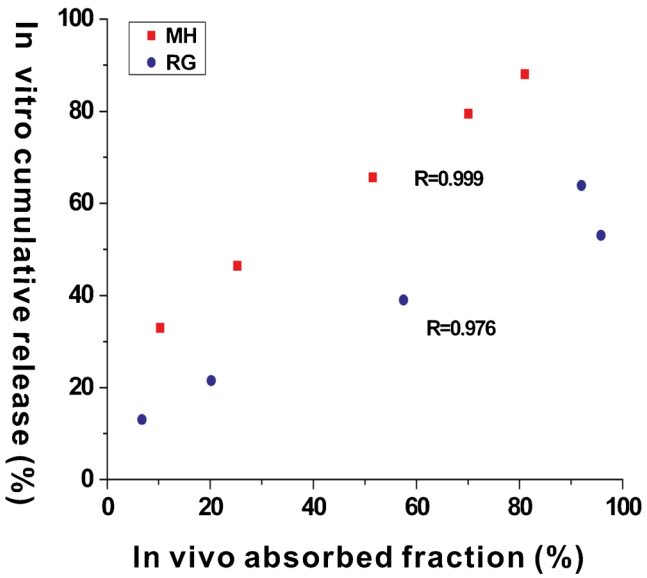

Fig. 6. *In vitro–in vivo* correlation of absorption profile and percent release *in vitro* at the same time points for the MH–RG sustained release bilayer tablets

establishment of *in vitro–in vivo* correlation (IVIVC) (33). Herein, a level A IVIVC was performed using the percentage of drug release from optimized formulation of sustained release bilayer tablets in water *vs*. the fraction of *in vivo* absorbed data. A good linear regression relationship was obtained between the percentage of drug release and the fraction of absorption since the values of correlation coefficient (R) of both drugs were greater than 0.9 (Fig. 6), therefore suggesting that the *in vivo* performance could be well foretold by the test of *in vitro* release.

CONCLUSIONS

The bilayer tablets for continuously releasing MH and RG simultaneously were successfully developed, in which the preparation process was simple and the scaling up were easy. The tablets could continuously deliver both MH and RG for 12 h, and a better *in vivo* behavior with a steady drug concentration in plasma, markedly reduced maximum of plasma concentration, and prolonged time to reach peak concentration and perfect absorption was obtained. Moreover, the *in vitro* and *in vivo* release of the two drugs was synchronic, having a potential to enhance the synthetic effect for T2D therapy. Summarily, the present bilayer matrix tablets could simultaneously release the two drugs with a sustained release profile, thereby prolonging the actions for T2D treatment and producing better health outcomes.

ACKNOWLEDGMENTS

This work was supported by the Creation of Major New Drugs national major projects (2011ZX09202-101-24, 2012ZX09202101-008, 2014ZX09507004-001), and the "333" High Level Talents Cultivation Project of Jiangsu Province.

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