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Preparation, in vitro and *in vivo* evaluation of a novel mitiglinide microemulsions

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ABSTRACT

This study aimed to prepare an o/w mitiglinide microemulsion (MTGME) to improve the drug solubility and bioavailability. The formulation of o/w MTGME was optimized by the solubility study of drug, pseudo-ternary phase diagram and Box-Behnken design successively. MTGME was characterized by dynamic laser light scattering (DLS), zeta potential and transmission electron microscopy (TEM), moreover, the storage stability, pharmacodynamics and pharmacokinetics were investigated. The optimal prescription for MTGME consisted of Maisine 35–1 (oil), Cremophor EL (surfactant) and propylene glycol (PG, cosurfactant). MTGME with a spherical dimension of 58.1 ± 5.86 nm was stable when stored at 4 °C for 3 months. The blood glucose levers (BGL) of diabetic mice were uniformly and significantly decreased by intragastric (*i.g.*) administration of 1–4 mg/kg MTGME, in which BGL (*i.g.* 4 mg/kg MTGME) was reduced by 69% during 24 h. The pharmacokinetics study of MTGME (*i.g.*, 20 mg/kg) in Wistar rats showed higher plasma drug concentration (C_{max}, 2.9 folds), larger area under curve (AUC, 4.6 folds) and oral bioavailability than those of MTG suspensions. Generally, the MTGME (o/w) showed good effect on controlling hyperglycemia. Therefore, microemulsion can be used as an effective oral drug delivery system to improve the bioavailability of MTG.

1. Introduction

It has been reported that about 40% drugs currently on the market possess poor water solubility characteristics (Jermain et al., 2018). The solubility and permeability of drug are important factors to achieve therapeutic effect. Therefore, many methods can be used to improve the solubility of hydrophobic drugs, such as solid dispersion, liposome, microemulsion and eutectic technology (Krishnaiah, 2010). Microemulsion (ME) or nanoemulsion (NE) is composed of oil phase, water phase, surfactant and co-surfactant. It shows Tyndall effect and possesses thermodynamical stability. As a drug delivery system, ME or NE has many advantages, including protecting unstable drugs, sustaining drug release, enhancing solubility and bioavailability of drug (He et al., 2010; Xavier-Junior et al., 2017). For example, the oral bioavailability of ibuprofen NE was 2.2 times higher than that of the control formulation (Anuar et al., 2020). Asenapine maleate was made into a nasal mucosal adhesion NE to improve its poor oral bioavailability, and the pharmacokinetic study of rats showed that intranasal administration of the NE increased significantly the concentration of drug in the brain (Kumbhar et al., 2020). Trans-resveratrol essential oil ME could improve successfully drug solubility (Lv et al., 2018). In addition, the microemulsion technology has been used to improve the solubility and bioavailability of piroxicam and glimepiride (Xing et al., 2016; Li et al., 2016).

Mitiglinide calcium (MTG, Fig. 1A), a hydrophobic and weakly acidic drug, is a type of glinides drug and fast-acting insulinotropic agent (Hadi et al., 2012). It induces less hypoglycemia than sulfonylureas and is a safe and effective treatment drug in the early stages of diabetes (Wang et al., 2012; Shigeto et al., 2007). Moreover, frequent administration is required due to absorbing MTG through the stomach with short onset (Mahmoud et al., 2018). Therefore, ensuring the drug efficacy and reducing the administration frequency are the key direction for MTG formulation design. It had been reported that the C_{max} and $AUC_{0-\infty}$ of MTG microsponge were 1.92 and 20.68 times higher than those of commercially available tablets. Meanwhile, mean residence time (MRT) and $t_{1/2}$ were prolonged by 7.22 and 7.97 times, respectively. Gastroretentive microsponge or in situ gel could prolong the release of MTG effectively (Mahmoud et al., 2018; Mahmoud et al., 2019). Our group

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had once reported to improve oral bioavailability of poorly water-soluble glimepiride by utilizing microemulsion technique (Li et al., 2016). Moreover, no related report on the MTGME preparation has been found so far.

The main purpose of this study was to design MTGME to accelerate drug absorption and improve its bioavailability. Therefore, pseudo ternary phase diagrams and Box-Behnken design were utilized to optimize MTGME formulation, and MTGME was characterized by DLS, zeta potential and TEM. The storage stability of MTGME was assessed and the BGL fluctuation of diabetic mice after administrated MTGME was studied. Moreover, the pharmacokinetic study of MTGME *in vivo* was also performed.

2. Materials and methods

2.1. Materials

MTG was purchased from Wuhan Long-distance Technology Development Co., Ltd. (China). Labrafac lipophile WL1349, Labrafil M 1944CS, Plurol® Oleique CC497, Lauroglycol 90, Capryol 90, Maisine 35-1 and Transcutol HP were provided by Cattefosse (France). Cremophor RH40 and Cremophor EL were supplied by BASF (Germany). Isopropanol, n-butyl alcohol, PG and other reagents were purchased from Xingyang Chemical Co. Ltd. (China). Wistar rats and BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The animal experiments were in compliance with the license of the National Laboratory Animal Use Law of the People's Republic of China and the study was performed according to the Hebei University Institutional Animal Care and Use Committee (IACUC) guidelines (Approval No. IACUC-2021XS042).

2.2. Preparation of MTGME

Excess MTG was added into the different oil phases, surfactants and co-surfactants respectively and then placed in an oscillator at (37 ± 0.2) °C for 3 days in the dark. After centrifuged at 12000 rpm for 10 min, the supernatant was taken out and the contents of MTG in different solvents were determined by High Performance Liquid Chromatography (HPLC). The analysis was performed on a Hypersil BDS C18 column (4.6 × 250 mm, 5 μm) with a flow rate of 1 mL/min. The mobile phase was acetonitrile–water (55:45, *v/v*, pH = 2.4) and the UV detection wavelength was 210 nm.

Based on the solubility of MTG in different solvents, the pseudo-ternary phase diagram was constructed. The surfactant mixture (Sm) was obtained by mixed uniformly the surfactant and co-surfactant at specific weight ratios (Km) of 3:1, 5:2, 2:1, 1:1 and 1:2 (*w/w*). The oil phase at the ratio of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (*w/w*) was added into Sm, subsequently, the distilled water was dropped slowly under magnetic stirring to obtain ME. During the preparation process of ME, the critical point of the turbidity and clarification of the solution were observed and the water consumption mass was recorded. According to the respective mass percentages of oil, water, and Sm at the critical point, the pseudo-ternary phase diagram was mapped to confirm the ME area (Zainuddin et al., 2021; Yin et al., 2009). MTGME was prepared by adding excess MTG to ME and incubated with shaking at 37 °C for 72 h in the dark. After centrifuged at 12000 rpm for 10 min, the supernatant was taken out and the concentration of MTG in ME was measured by HPLC.

To optimize the ME formulation for the high solubility of MTG, the 3 factors and 3 levels Box-Behnken design response surface method was used and the proportion of oil (X_1), Sm (X_2), and water (X_3) was identified as key factors to investigate. The content of MTG was used as an index to evaluate the ME formula and the result was analyzed by Design Expert software.

2.3. Characterization of MTGME

The particle size and zeta potential of optimized ME or MTGME were determined in triplicate by using DLS (Delsa TM Nano zeta potentiometer, Beckman Coulter, USA). To image the morphology of MTGME and

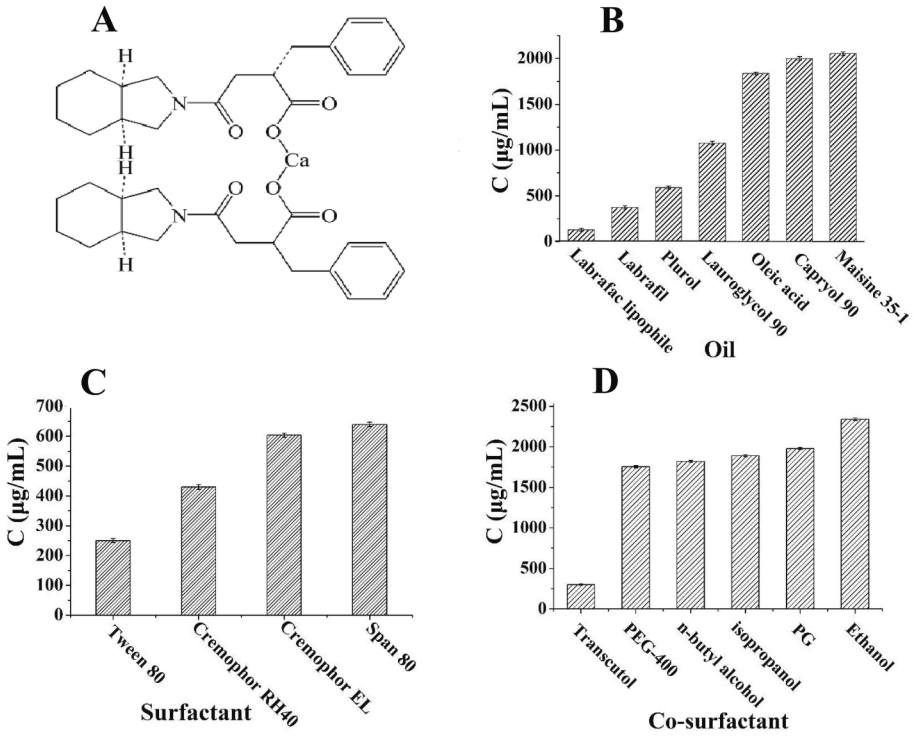

Fig. 1. (**A**) The molecule structure of MTG; (**B**) The solubility of MTG in different oil phases; (**C**) The solubility of MTG in different surfactants; (**D**) The solubility of MTG in different co-surfactants. The value of solubility is the average of three samples.

ME, the air-dried samples on the copper mesh was dyed by 2% phosphotungstic acid. After the grids were dried, the morphology of MTGME and ME was observed by TEM (JEM-100XS, JEOL, Japan). To investigate the stability of MTGME and ME, the samples were sealed and stored at 4 °C for 3 months. The changes of appearance and drug content in the samples were evaluated (Monton et al., 2020). The dye method was used to identify the type of ME. The water-soluble dye methylene blue and the oil-soluble dye Sudan III were added to ME samples, respectively. Based on the diffusion velocity of dye in the ME, the type of o/w or w/o was measured.

2.4. In vitro release of MTGME

The release of MTG from ME was determined by dialysis method (Li et al., 2016; Sedyakina et al., 2019). Disperse 2 mg of MTG in 1 mL of distilled water or MTGMEs containing 2 mg of MTG was placed in an 8–14 KDa dialysis bag, respectively. The dialysis bag was then submerged in 20 mL of fresh PBS with different pH (1.2, 6.8, 7.4) at (37 ± 2) ℃ in a shaker at 100 r/min. At a specific time (0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48 h), all release medium was taken out and replaced with the same fresh buffer. The drug content at each time point was determined by HPLC. The cumulative release rate (Q%) of drug was calculated according to Eq. (1).

$$Q\% = \frac{VCn + \sum_{i=1}^{n-1} CiVi}{W0} \times 100\% \quad (1)$$

2.5. Pharmacokinetics study in rat

After fasted for 12 h before the experiment, healthy female Wistar rats aged 6–8 weeks (180–200 g) were randomly divided into two groups (n = 3) and given intragastric administration of MTG suspensions and MTGMEs at a dose of 20 mg/kg MTG, respectively. 0.5 mL of blood was taken from the orbit of the rats at different time points (0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 h) (Zhao et al., 2021). The plasma was separated by centrifugated at 12,000 r/min for 10 min. 0.6 mL of methanol-chloroform (1:4, *v/v*) was added to 200 μL plasma and homogenized on a vortex mixer for 5 min. Followed by centrifuged at 12,000 r/min for 10 min, the organic layer was removed and evaporated under nitrogen gas flow. Redissolved the residue with the mobile phase and centrifuged at 6000 r/min for 5 min, 20 μL sample was analyzed by HPLC and the concentration of MTG was measured. The main pharmacokinetic parameters were calculated by DAS 2.0.

2.6. Pharmacodynamics study in mice

BALB/c mice were used to induce diabetic model by intraperitoneal injection of alloxan saline solution at a dose of 180 mg/kg. The control group was treated with normal saline. After the injection, all animals took food and water freely, meanwhile, the food intake and body weight of the mice were recorded every day. On the 3rd and 7th day, the blood glucose of the experimental mice was measured. When the BGL was greater than 14.5 mmol/L, mice were considered as diabetic (Lartey et al., 2021; Cao et al., 2016). Diabetic mice were divided into 3 groups at random (n = 5). The hypoglycemic effects of MTGME by intragastric administration at high, medium and low dose (4, 2 and 1 mg/kg) were investigated. After administration, blood was taken from the tail at the specified time points (0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 h), and BGL was measured by Glucometer (Sannuo Anhuai blood glucose meter, China).

2.7. Statistical analysis

The data are expressed as mean ± SD. Student's *t*-test is used for statistical analysis and $p < 0.05$ is considered statistically different.

3. Results

3.1. Solubility study of MTG

MTG is soluble in methanol or ethanol, and insoluble in water, ether, hydrochloric acid (0.1 mol/L) and sodium hydroxide (0.1 mol/L). The solubility of MTG in water is about 86 μg/mL. Fig. 1B shows the solubility of MTG in seven different oil phases. The type of oil phase had a greater influence on the solubility of MTG. The solubility of MTG in Capryol 90 and Maisine 35–1 were better than those of other oil phases, reaching 2006.67 μg/mL and 2057.50 μg/mL, respectively. These two oils has been commonly found in the ME literature. For example, Capryol 90 and Maisine 35–1 were used as the oil phase to prepare myricetin /or glibenclamide ME and w/o metformin hydrochloride ME, respectively (Qian et al., 2017; Shakeel et al., 2013; Li et al., 2014). Thus, this experiment tried to use Capryol 90 /or Maisine 35-1 as oil phase for preparing MTGME. Fig. 1C shows the solubility of MTG in four different surfactants. The solubility of MTG in Span-80 or Cremophor EL reached to 639.59 μg/mL and 623.67 μg/mL, respectively. Span-80 and Cremophor EL are also commonly used as surfactants for ME. The mixture of Span-80 and α-tocopheryl polyethylene glycol succinate (TPGS) as a surfactant could improve the in vitro release behavior of paclitaxel (Gandhi et al., 2021). Cremophor EL was used to prepare ME for investigating the effects on human blood components and coagulation function (Zhang et al., 2019). Therefore, Span-80 and Cremophor EL were chosen as alternative surfactant. Fig. 1D shows the solubility of MTG in five different co-surfactants. The solubility of MTG in PG reached to 2177.13 μg/mL, only lower than in ethanol. However, ethanol is easy to volatilize. PG has a salting-out effect and can be incorporated into the surfactant layer to increase interfacial fluidity. In addition, it is also easily soluble in water and can reduce the polarity of water. Hence, PG is suitable as a co-surfactant to develop the MTGME (Gharbavi et al., 2019).

3.2. Pseudo ternary phase diagram

Pseudo ternary phase diagrams were utilized to optimize the compositional phase and observe the thermodynamical stability of ME. Using Capryol 90 or Maisine 35-1 as the oil phase and Span-80 as the surfactant, ME could not form. It is reported that Span-80 always combines other surfactant to prepare ME (Gandhi et al., 2021; Ghorbanzadeh et al., 2019). Thus, Cremophor EL was chosen as surfactant due to its good solubility for MTG. The pseudo ternary phase diagrams of Maisine 35-1, Cremophor EL/PG (Km) and water (Fig. 2A–E) were constructed, in which the shaded area was the ME area. When Km was 5:2, the largest ME area appeared. The high surfactant content could reduce the particle size of the ME (Kommana et al., 2020). The phase diagrams of Capryol 90 and Cremophor EL/PG systems (Km = 5:2, 2:1, 1:1) were shown in Fig. 2F–H and the ME area fixed Km 5:2 was also the largest.

Based on the results of phase diagrams, MTGME was prepared. The surfactant and co-surfactant with the optimal Km value were mixed uniformly. Adding appropriate oil phase and distilled water in sequence under stirring, the transparent ME with light blue opalescent spontaneously formed. Subsequently, the excess amount of MTG was mixed with blank ME to prepare MTGME. The content of drug loading in different MTGME formula was determined and the results were shown in Table 1. It could be seen from Table 1 that the content of MTG in F-2 was the largest. Therefore, Maisine 35-1 and Cremophor EL/PG (Km = 5:2) system was selected for the further optimization.

3.3. Response surface methodology

The Box-Behnken design with 3 factors and 3 levels was utilized to optimize the MTGME formulations using the drug content as an evaluation index. The factor level and the drug content in different formulations are shown in Table 2. The contour plots and 3D response surface

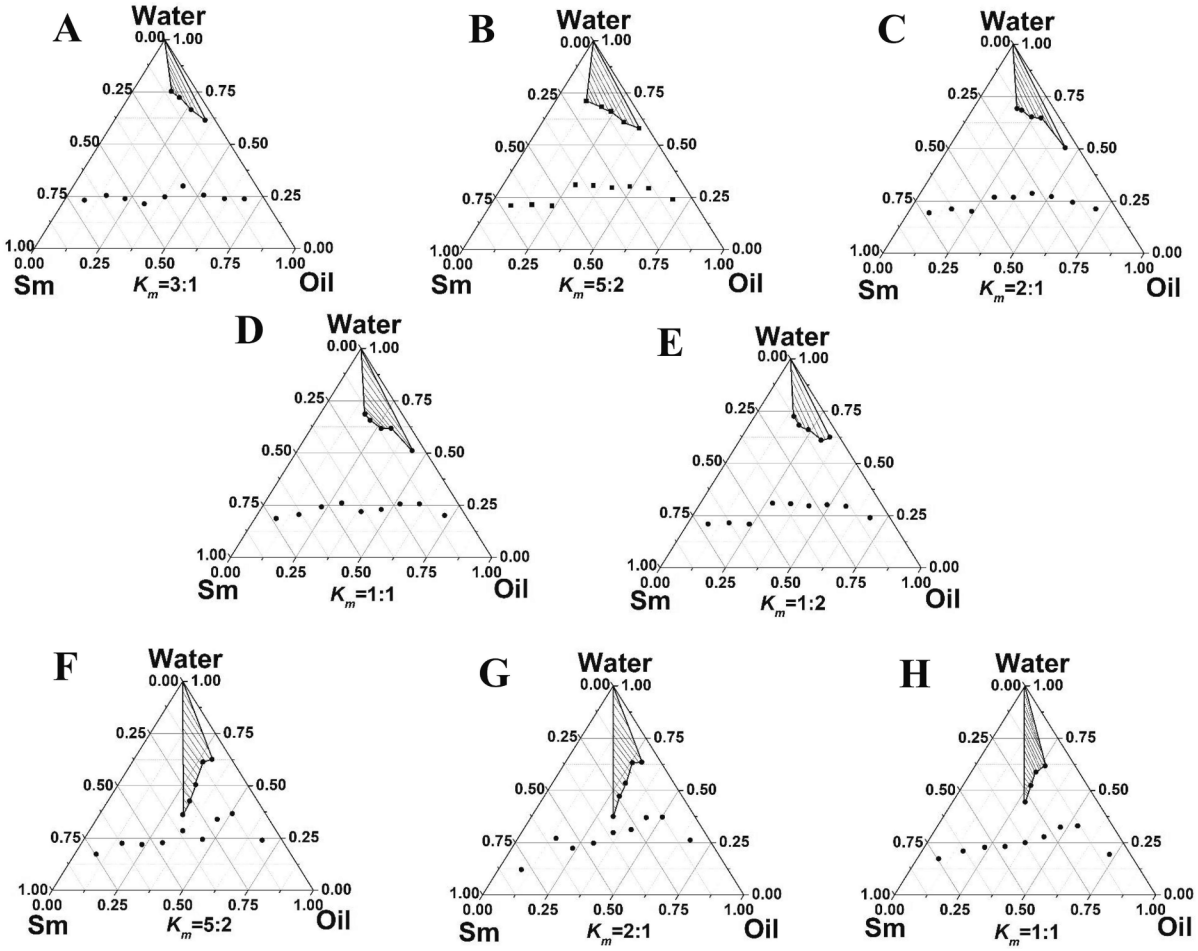

Fig. 2. The pseudo ternary diagrams composed of Maisine 35-1, Cremophor EL/PG (Km = 3:1, 5:2, 2:1, 1:1, 1:2) and distilled water; The pseudo ternary diagrams composed of Capryol 90, Cremophor EL/PG (Km = 5:2, 2:1, 1:1) and distilled water.

Table 1
MTG content of the different formulations (n = 3).

Formulation	Km	Oil	S/Co-S	Oil:Sm: Water	MTG (mg/mL)
F-1	3:1	Maisine 35–1	Cremophor EL/PG	10:22:68	1.57 ± 0.17
F-2	5:2	Maisine 35–1	Cremophor EL/PG	7:29:64	2.63 ± 0.12
F-3	2:1	Maisine 35–1	Cremophor EL/PG	10:24:66	2.05 ± 0.15
F-4	1:1	Maisine 35–1	Cremophor EL/PG	10:23:67	1.91 ± 0.25
F-5	1:2	Maisine 35–1	Cremophor EL/PG	9:21:70	1.76 ± 0.18
F-6	5:2	Capryol 90	Cremophor EL/PG	14:21:65	2.31 ± 0.16
F-7	2:1	Capryol 90	Cremophor EL/PG	13:20:67	2.02 ± 0.17
F-8	1:1	Capryol 90	Cremophor EL/PG	11:26:62	2.30 ± 0.12

plots are shown in Fig. 3.

It could be seen from Table 2 that the drug content of MTGME ranged from 1.63 to 3.37 mg/mL and the highest content of MTG was appeared in F-8. Design-Expert 8.0.6 software was used to perform binomial fitting analysis, and the regression equation was $Y = 2.07–0.33X_1-0.26X_2-0.20X_3-0.19X_1X_2-0.055X_1X_3-0.37X_2X_3 + 0.036X_1^2 + 0.34X_2^2 + 0.54X_3^2$. The correlation coefficient (R^2) and adjusted coefficient (R_{adj}^2) of the quadratic equation were 0.9812 and 0.9570, respectively,

Table 2
Variables and results in the Box-Behnken design.

Formulation	Independent variables			MTG concentration/(mg/mL)
	Oil/X_1	Sm/X_2	Water/X_3	
F-1	−1	−1	0	2.89
F-2	1	−1	0	2.58
F-3	−1	0	−1	3.02
F-4	1	0	−1	2.48
F-5	0	0	0	2.03
F-6	0	0	0	2.05
F-7	−1	1	0	2.68
F-8	0	1	−1	3.37
F-9	0	0	0	2.11
F-10	0	0	0	2.05
F-11	−1	0	1	2.92
F-12	1	0	1	2.16
F-13	1	1	0	1.63
F-14	0	1	1	2.05
F-15	0	−1	1	3.26
F-16	0	0	0	2.11
F-17	0	−1	−1	3.11

Independent variables	Levels		
	Low (−1)	Medium (0)	High (+1)
X_1 = oil (%)	3	8	13
X_2 = Sm (%)	15	23	31
X_3 = water (%)	60	68	76

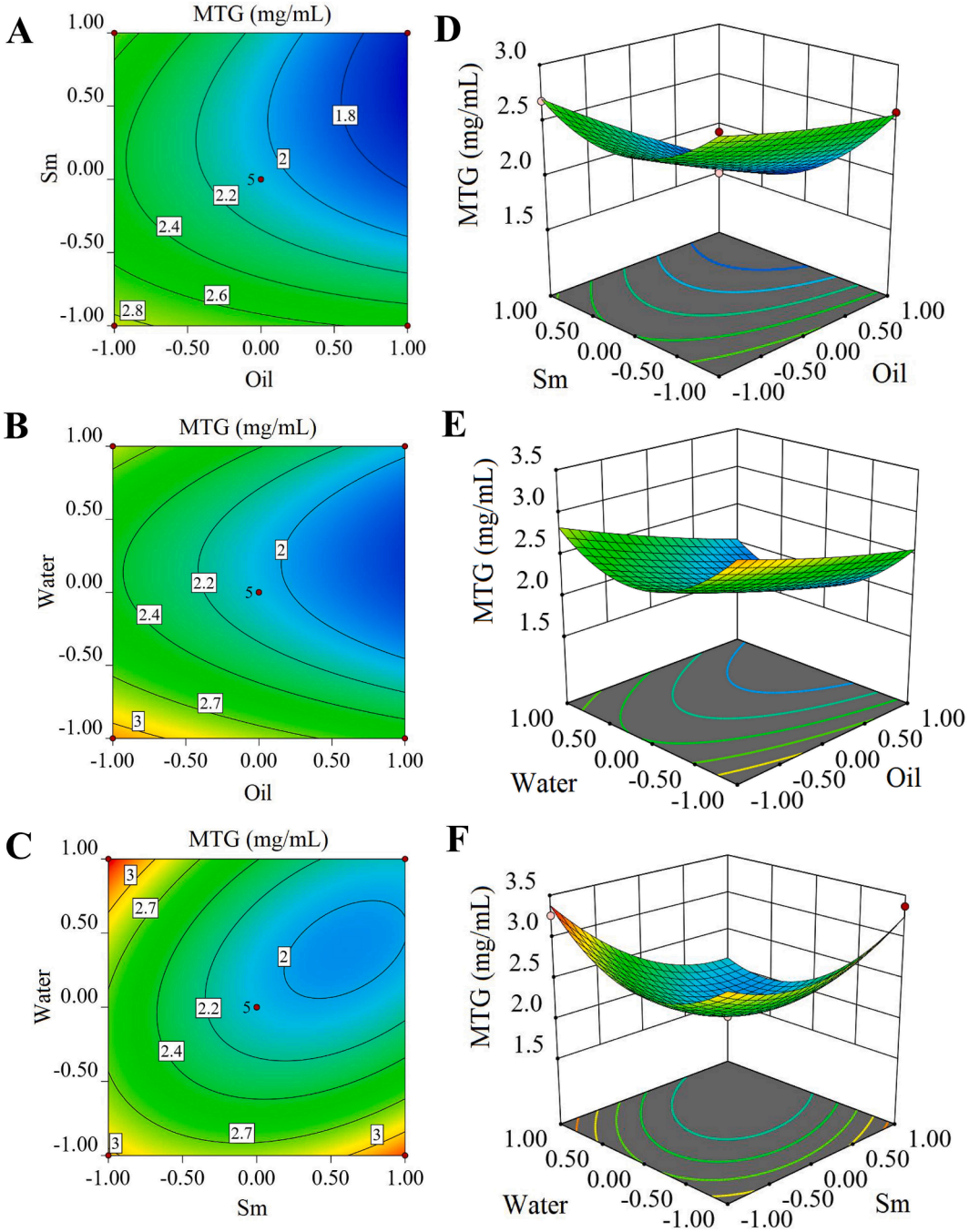

Fig. 3. Contour plot (**A–C**) and corresponding response surface plot (**D–F**) showing the effect of oil, Sm, and water on the concentration of MTG in ME.

indicating that the model equation had a good fit. Moreover, the coefficient of variation (CV%) was 4.33%, which was low and the model was available. Analysis of variance and statistical test results showed that the model P-value was extremely significant ($P < 0.0001$). The model parameters X_1, X_2, X_3, X_1X_2, X_2X_3, X_2^2 and X_3^2 were all significant ($P < 0.05$), and especially X_1 and X_3^2 were extremely significant ($P < 0.001$). Therefore, the model could be used to analyze the optimization prescription of MTGME.

As shown in Fig. 3, the effect of the independent variables was evaluated and the formulation was optimized. It could be seen that the drug contents first decreased and then increased with increasing the amount of the oil (X_1), Sm (X_2) and water (X_3), in which the oil phase played a key role in affecting the MTG content. When the amount of oil phase was higher, the increase of Sm and water would lead to the decrease of MTG content (Fig. 3A, B, D and E). Moreover, the higher value of Sm caused the more water and the lower MTG content (Fig. 3C and F).

The model-predicted optimal values of the three variables were X_1 = 8%, X_2 = 31%, X_3 = 61%. The maximum predicted concentration of MTG in ME was 3.30 mg/mL. To confirm the optimized conditions, three batches of optimal MTGME was prepared. The drug content of MTGME measured by HPLC was 3.34 ± 0.21 mg/mL, indicating that the optimal MTGME was obtained by this model.

3.4. Characterization of MTGME

Fig. 4 shows TEM image of ME and MTGME. It could be seen from that the shapes of ME and MTGME were both close to sphere. The size

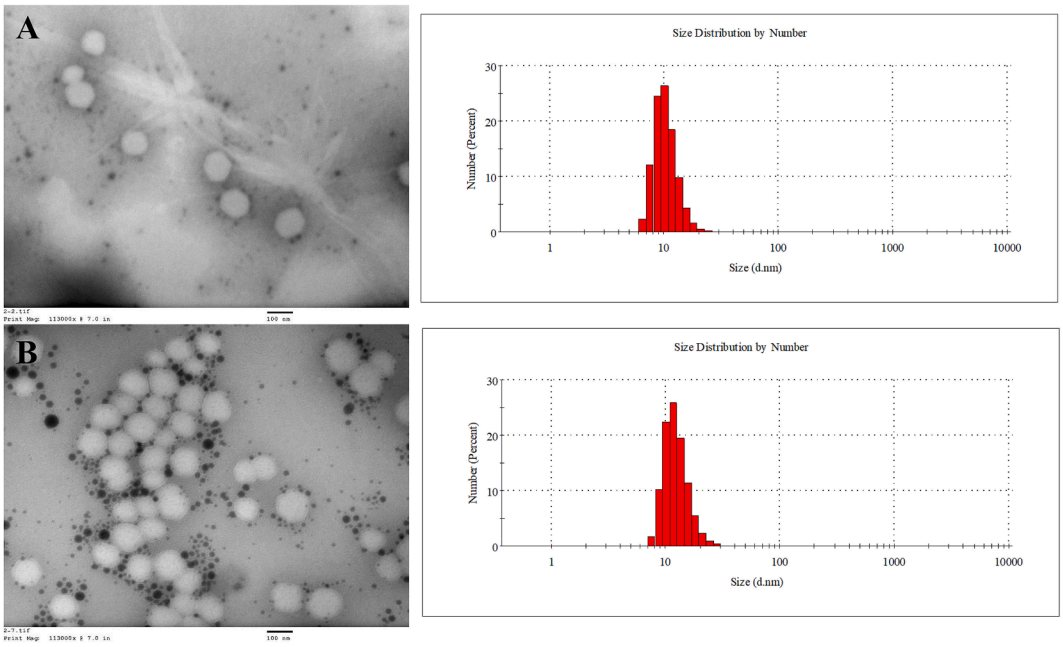

Fig. 4. The morphology and its size distribution (**A**) ME and (**B**) MTGME, and a scale bar is 100 nm. [a]MTGME sealed storage at 4 °C for 3 months.

and particle dispersion index (PDI) values of ME and MTGME are shown in Fig. 4 and Table 3. The particle size of ME and MTGME (PDI value < 0.3) were 53.6 ± 1.61 nm and 58.1 ± 5.86 nm, respectively. There was no significant change between ME and MTGME. Moreover, the particle size of MTGME stored at 4 °C for 3 months did not vary significantly (59.5 ± 8.06 nm), indicating that MTGME was stable during storage stage. It was interesting that the size of blank ME became larger when it was diluted by 50 times or 100 times. However, it still appeared light blue opalescence, no turbidity or delamination. The dyeing experiment showed that the diffusion rate of blue (methylene blue) in the ME was greater than that of red (Sudan III), indicating that the ME was o/w type.

3.5. *In vitro release of MTGME*

Fig. 5 shows the in vitro release profiles of MTGME and MTG suspensions in different pH media. The release amount of MTGME in different media was better than that of the MTG suspensions, indicating that MTGME could significantly improve the dissolution of the drug. The optimal drug release from MTGME was obtained at pH 7.4 medium (Fig. 5C), and the cumulative drug release reached 80% within 8 h, which was 2.6 times than MTG suspension. Meanwhile, the drug release amount of MTGME in different pH media (1.2, 6.8, 7.4) reached 90%, 97% and 100% within 48 h, respectively. Therefore, the MTGME improved the solubility and dissolution of MTG.

Table 3
The size, PDI and zeta potential of ME and MTGME.

Sample	Diluted multiples	Diameter (nm)	PDI	Zeta potential (mV)
ME	0	53.6 ± 1.61	0.282 ± 0.003	−1.38 ± 0.32
	50	84.6 ± 9.54	0.227 ± 0.036	−0.36 ± 0.95
	100	87.1 ± 3.97	0.210 ± 0.022	0.16 ± 0.22
MTGME	0	58.1 ± 5.86	0.278 ± 0.055	0.63 ± 1.73
MTGME[a]	0	59.5 ± 8.06	0.216 ± 0.025	0.72 ± 1.23

[a] MTGME sealed storage at 4 °C for 3 months.

3.6. *Study on the pharmacokinetics and pharmacodynamics of MTGME in vivo*

Wistar rats were given intragastric administration of MTG suspensions and MTGME at a dose of 20 mg/kg of MTG, respectively. The content of drug in blood was determined by HPLC. The determination of MTG was not disturbed by plasma and the retention time of drug was 7.9 min. The linear equation of MTG standard pharmacokinetic curve was $y = 2.54 \times 10^5 x + 3.33 \times 10^6$ ($R^2 = 0.9974$, y stands for A_{abs}; x stands for drug concentration, μg/mL.). The linear relationship was good in the concentration range of 0.01–50 μg/mL. The RSD for the inter- and intra-day was 3.52% and 5.02% (n = 5), respectively. The average recovery of low, medium and high concentration was in the range of 95.5%-100.8%, which met the determination requirements of biological sample (Ramadon et al., 2020). The plasma concentration–time curve is shown in Fig. 6A, and the pharmacokinetic parameter (Table 4) was obtained by DAS 2.0 software. The pharmacokinetic profile of the drug in both the MTG suspension and the MTGME followed a two-compartment model. The AUC value of MTG suspension and MTGME in rats was 28.75 ± 4.01 mg·h/L and 133.01 ± 5.47 mg.h/L, respectively, indicating the bioavailability of MTG was significantly improved by ME. The maximum plasma concentration of MTGME (C_{max}, 23.96 ± 2.37 mg/L) achieved at 1 h and the half-life ($T_{1/2}$) was prolonged, which were about 2.9 and 4.5 times than that of MTG suspension, respectively. Therefore, the above results showed that MTGME could enhance the bioavailability of MTG effectively.

Fig. 6B and 6C show the change of the body weight and food intake of diabetic mice during experimental process. Compared with the mice in the control group, diabetic mice showed lower weight and higher food intake, which was in line with the symptoms of diabetic mice (Cao et al., 2016). Considering the viscosity of MTGME, the intragastric (*i.g.*) administration dose of MTGME was set to 4, 2 and 1 mg/kg, respectively. As shown in Fig. 6D, the BGL of diabetic mice after intragastric (*i.g.*) administration of MTGME consistently decreased to below 14 mmol/L within 24 h. Moreover, high dose of MTGME (4 mg/kg) improved the hypoglycemic effect and decreased BGL of diabetic mice from 26.16 ± 6.37 to 8.23 ± 1.91 mmol/L during 24 h. The results demonstrated that MTGME had the potential to control hyperglycemia.

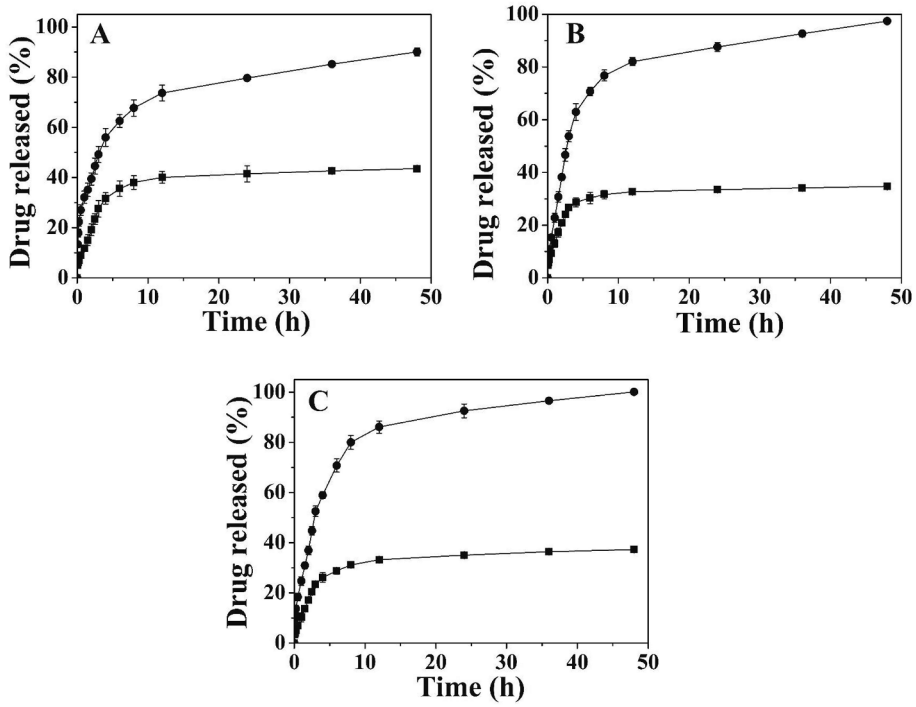

Fig. 5. Cumulative release curve of MTGME (-●-) and MTG suspension (-■-) at 37℃, (**A**) pH 1.2, (**B**) pH 6.8 and (**C**) pH 7.4.

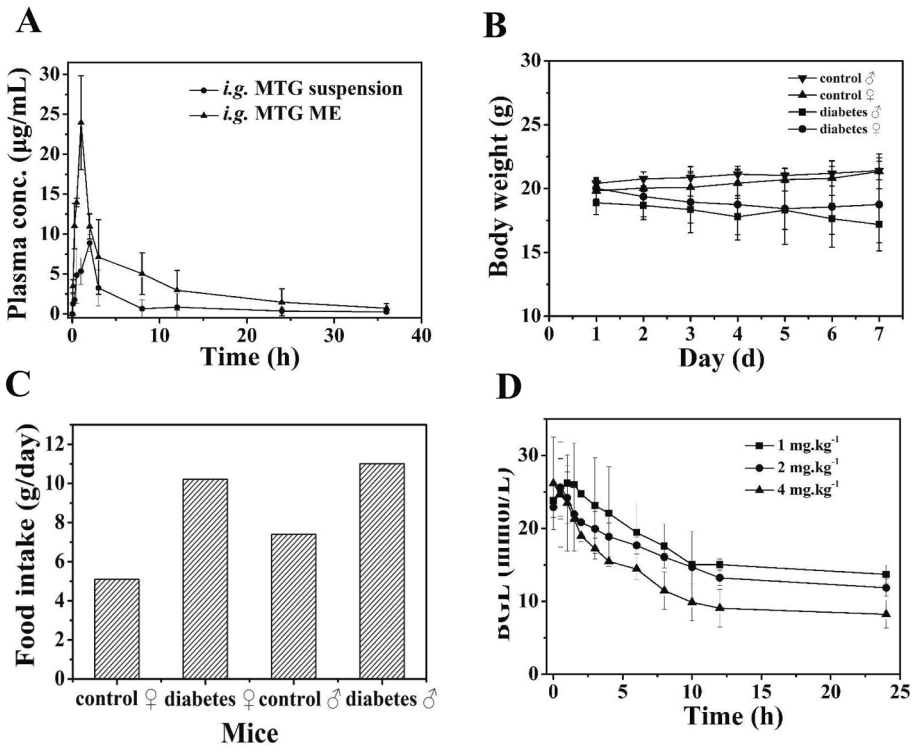

Fig. 6. (**A**) Plasma concentration–time curves after administration at a dose of 20 mg/kg (n = 3); (**B**) Control of weight loss and (**C**) Increase of food intake; (**D**) Dose dependent response.

4. Discussion

ME, consisted of oil, water, surfactant and co-surfactant phase, is an isotropic and thermodynamically stable transparent system. Reducing the droplet size to the nanometer level is an effective way to increase the dispersed phase surface area of the insoluble drug. ME (droplet size < 500 nm) can be formed under mild stir conditions and it can promote the drug absorption by improving drug dissolution and diffusion, accelerating intestinal lymph transport of drugs, preventing enzymatic hydrolysis and inhibiting the efflux of P-glycoprotein (P-gp) (Zhao et al., 2013). This approach has been proved to be effective for BCS Class II drugs, such as glimepiride and cyclosporin A (Li et al., 2016; Gao et al.,

Table 4
Pharmacokinetic parameters of MTG suspension and MTGME in rats (n = 3).

PK parameters	*i.g.* administration	
	MTG suspension	MTGME
$T_{1/2}$ (h)	1.17 ± 0.73	5.18 ± 2.64
CL/F (L/h/kg)	0.57 ± 0.21	0.18 ± 0.09
T_{max} (h)	2	1
C_{max} (mg/L)	8.23 ± 0.95	23.96 ± 2.37
AUC_{0-48} (mg h/L)	28.75 ± 4.01	133.01 ± 5.47

$T_{1/2}$ (h): elimination half-life; CL/F: total plasma clearance of oral; T_{max}: time to reach C_{max}; C_{max}: maximum concentration; AUC_{0-48}: area under the curve of a plasma concentration versus time profile.

1998). Single ME is usually divided into oil-in-water (o/w) and water-in-oil (w/o) types. It has been reported that the cell membrane permeability of w/o type is higher than that of o/w type. Drug-loaded MEs are transported through lymph, which is the main factor to improve the bioavailability of oral drug. Moreover, different types of oral ME could significantly affect the degree of lymphatic absorption (Tang et al., 2013). In our study, pseudo ternary phase diagrams were utilized to optimize the compositional phase and observe the thermodynamical stability of MEs, and the MTGME formulations were further optimized by the Box-Behnken design. MTGME, as an o/w type, exhibited light blue opalescence and was stable during storage stage.

Usually, MEs have the potential to be drug carriers because they can increase the solubility of certain drugs and significantly improve their oral bioavailability. When ME is diluted or dispersed in gastrointestinal tract (GIT), attention should be paid to the deposition of drugs. Long-term use of MEs containing large amounts of surfactants may irritate the GIT. Therefore, prescription ingredient selection should carefully consider the safety of surfactant carriers and test ways to reduce their dosage in the formulation. The choice of surfactants also greatly affects the toxicity of MEs for oral delivery of drugs. In general, polyethoxylated vegetable oils and polysorbates are less toxic than single-chain surfactants, and esters are less toxic than ethers. Non-ionic surfactants are generally considered to be acceptable for oral administration. The oral and intravenous LD50 values of most non-ionic surfactants exceed 50 and 5 g/kg, respectively. The marketed HIV protease inhibitor products (Agenerase and Norvir) contain a considerable quantity of surfactants in each capsule, and several capsules are administered 2–4 times daily, indicating that patients ingest Cremophor of 2–3 g one day. It is generally believed that in this case, the benefits of clinical indications outweigh the risks, hence, such formulations are still acceptable overall (He et al., 2010). Commercially available MTG tablets were taken 5 min before meals (adults 10 mg 3 times a day). The dosage can be adjusted according to the patient's treatment effect. In our study, the maximum predicted concentration of MTG in ME was 3.30 mg/mL (Oil:Sm:Water = 8%:31%:61%, Sm = Cremophor EL:PG = 5:2, Cremophor EL $\rho = 1.05$ g/cm^3), and the Cremophor EL in the ME was calculated according to the daily dose (30 mg) of MTG tablet, containing about 2.1 g. Meanwhile, the oil (Maisine 35–1, ester, $\rho = 0.96$ g/cm^3) in oral was about 0.72 mL a day and <0.7 g. Therefore, the type and dosages of oil and surfactant phase used in MTGME prescription are safe. Moreover, the study on the pharmacokinetics and pharmacodynamics of MTGME *in vivo* showed that the preparation of MTGME could improve drug solubility in aqueous medium and simultaneously promote transmembrane permeation of MTG. Formulation design to improve oral bioavailability of poorly water-soluble drugs has been challenge for researchers. For oral administration, gastrointestinal digestion should be considered.

The reasons for the good bioavailability of MTGME *in vivo* were speculated as follows. Firstly, due to their nano scale size, larger surface area and penetration-enhancing properties, MTGME could be easily dispersed in the duodenum and jejunum villis, and then be absorbed greatly by lymphoid system of intestine, which could bypass the liver first-pass metabolism. Moreover, it has been reported that the lymphatic absorption pathway provides an opportunity to improve the bioavailability of highly lipophilic drugs. In addition, lipid-based drug delivery systems consisting of alcohol esters of unsaturated long-chain fatty acids have been shown to improve the bioavailability of certain drugs by promoting lymphatic absorption pathways (Griffin and O'driscoll, 2006). Secondly, MTGME could prevent drug rapid clearance from systemic circulation and resist physicochemical or enzymatic degradation. Finally, oil phase and surfactant in the ME could be benefit for opening the tight junctions between cells (Li et al., 2016; Alkrad et al., 2022). Therefore, using microemulsion technology, the solubility and bioavailability of MTG were enhanced significantly. Meanwhile, oral administration of MTGME may decrease administration frequency. It could improve the solubility of MTG and increase its bioavailability due to the facilitation of lymphatic absorption and avoiding the first pass metabolism.

5. Conclusion

In this work, o/w MTGME was developed and the optimized formulation was composed of maiisine 35-1, cremoophor EL and PG. In addition, *in vivo* pharmacokinetic and pharmacodynamic studies demonstrated that MTGME enhanced the bioavailability of MTG and decreased the BGL of diabetic mice. MTGME could act quickly and suppress hyperglycemia for a long time, thus increasing patients' compliance with the treatment for chronic disease. Therefore, the microemulsion can be an effective carrier for oral MTG administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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