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Preparation of rosiglitazone maleate sustained-release floating microspheres for improved bioavailability

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The object of this study was to prepare rosiglitazone maleate (RM) sustained-release floating microspheres and investigate their pharmacokinetics. RM microspheres were prepared with ethyl cellulose (EC) and octadecyl alcohol as the carrier materials by an emulsion-solvent diffusion method, and the properties of morphology *in vitro* floating capability, drug loading (DL), entrapment efficiency (EE), *in vitro* release and *in vivo* pharmacokinetics were investigated. The prepared microspheres had a completely spherical shape. The percentage of microspheres floating after 12 h was $(91.45 \pm 1.62)\%$, and the DL and EE were $(9.31 \pm 0.31)\%$ and $(89.55 \pm 1.65)\%$ respectively. Pharmacokinetic studies demonstrated that the RM floating microspheres were superior to commercial tablets in terms of the decrease in peak plasma concentration and maintenance of RM concentration in plasma. The area under the curve of plasma concentration–time (AUC) of the floating microspheres was equivalent to that of reference tablets. The results showed that floating microspheres are a feasible approach for the sustained-release preparation of drugs which have limited absorption sites in the upper small intestine.

1. Introduction

RM is an insulin-sensitizing oral thiazolidinedione used for treating patients with type 2 diabetes mellitus (Zhao 1999; Ding 2000). These drugs increase the sensitivity of target tissues (liver, muscle, and adipose tissue) to insulin, thereby reducing insulin resistance (Elbrecht et al. 1996). Studies of RM as monotherapy have demonstrated that the drug is well tolerated and reduces insulin resistance and blood glucose levels in patients with type 2 diabetes mellitus (Patel et al. 1999; Agrawal et al. 2003; Chapelsky et al. 2003). Preformulation researches show that RM is freely soluble in simulated gastrointestinal fluid (SGF) and that solubility decreases with increasing pH in the physiological range. Above pH 7, the solubility of the drug is very low, resulting in low and incomplete *in vitro* release, and the results show that RM has a narrow absorption window in the upper part of the gastrointestinal tract (GIT).

In this study, floating drug delivery systems (FDDS) were developed to overcome this limitation. This dosage form, with a prolonged residence time in the stomach, helps in the absorption of the drugs which are less soluble or unstable at alkaline pH and those which are absorbed from the upper gastrointestinal tract. It results in better control of fluctuations in plasma drug concentration in some cases (Brahma et al. 2000; Joseph et al. 2002; Ma et al. 2008; Kawashima et al. 1991) and retains the dosage form at the site of absorption, thus enhancing the bioavailability (El-Gibaly 2002; Sato et al. 2004).

At present, only a few RM floating drug delivery system formulations have been reported in the literature (El-Kamel et al. 2001). A multiunit floating drug delivery system of RM using Eudragit® RS100 as the coating polymer was developed (Kamila et al. 2009). RM floating microspheres prepared by a solvent diffusion evaporation method using ethyl cellulose and hydrox-

ypropylmethylcellulose (HPMC) were also developed (Rao et al. 2009). However, no *in vivo* pharmacokinetics have yet been reported for RM floating microspheres. As a drug with low solubility in the intestine, it is all the more necessary to determine its absorption in sustained-release delivery.

The objective of this research was to obtain better delivery of RM to the stomach and the proximal parts of the small intestine by increasing the mean residence time in the stomach. For this, gastro-retentive floating microspheres were prepared to prolong the gastric emptying and provide maximum drug exposure at the site of absorption. The properties of morphology, *in vitro* floating capability, DL, EE, *in vitro* release and *in vivo* pharmacokinetics of the floating microspheres prepared were investigated.

2. Investigations, results and discussion

2.1. Formulation optimization

The concentration of EC and octadecyl alcohol, the agitation speed and the concentration of poloxamer188 were chosen as the most influential factors (labeled as A, B, C, and D in Table 1). The four factors were investigated at three different levels taking the percentage of floating as an index. The L9 (3^4) orthogonal design was established as shown in Tables 1 and 2. The range, describing the relationship between the index and each factor, was chosen to select the optimum ingredient compositions reflecting the degree to which various factors affected the index. The ranking of the factors in this experiment was $B > A > C > D$, and the individual levels within each factor were ranked as: A: $2 > 3 > 1$; B: $3 > 2 > 1$; C: $1 > 2 > 3$; D: $2 > 3 > 1$. Based on the optimized results of the orthogonal design, the optimized formulation should be $A_2B_3C_1D_2$, i.e., formulation 6.

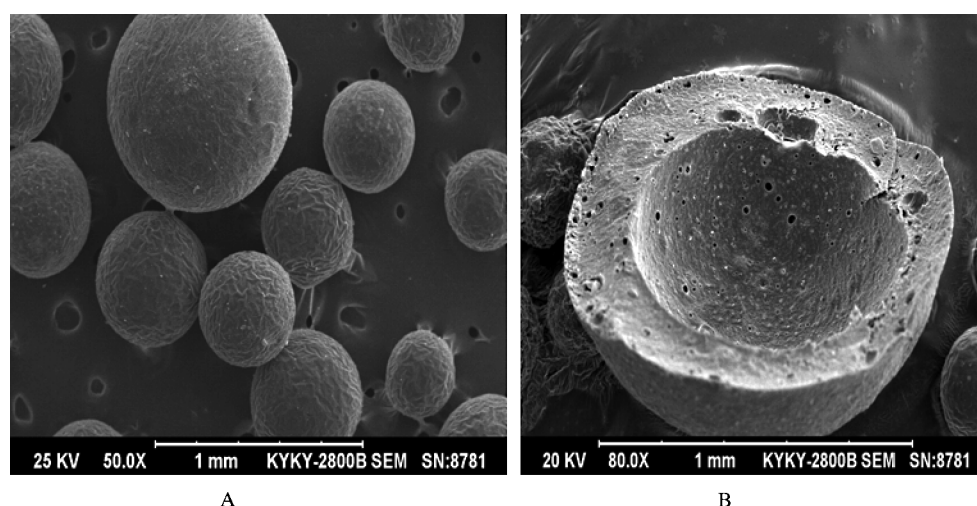


Fig. 1: A: SEM of RM floating microspheres. B: Cross-sections of RM floating microspheres

Table 1: Levels of experimental factors

Levers factors	A (% \cdot g \cdot ml $^{-1}$)	B (% \cdot g \cdot ml $^{-1}$)	C (rpm)	D (% \cdot g \cdot ml $^{-1}$)
1	6	1	100	0.4
2	10	5	300	0.8
3	14	10	600	1.2

All batches contained 0.2 g of RM, 7 ml acetic ether, 3 ml dichloromethane

The optimal formulation containing 0.2 g of RM, 1.0 g EC and 1.0 g octadecyl alcohol was dispersed in a mixture of 7 ml ethyl acetate and 3 ml dichloromethane (7:3, v/v) at room temperature. Dispersed droplets of the polymer solution of the drug were added to the aqueous phase containing 0.8% poloxamer 188 in water saturated ethyl acetate under stirring (100 rpm); finally phosphate buffer (pH 6.8) was added with a constant stirring rate that facilitated diffusion and evaporation of organic solvent.

2.2. Scanning electron microscopy (SEM)

SEM was conducted to investigate the morphology of the microspheres. Fig. 1A presents SEM micrographs of RM floating microspheres. The micrographs show regular spherical shapes. It is apparent that the microspheres have a smooth surface with no drug crystals and a few outer wrinkles due to the collapse of the microsphere wall during *in situ* drying in SEM analysis. The

Table 2: Orthogonal experiment design and results of floating test

Formulation	A	B	C	D	Percentage floating (%)
1	1	1	1	1	53.35
2	1	2	2	2	78.93
3	1	3	3	3	84.96
4	2	1	2	3	59.46
5	2	2	3	1	81.51
6	2	3	1	2	91.45
7	3	1	3	2	59.29
8	3	2	1	3	84.37
9	3	3	2	1	87.88
K ₁	72.41	57.37	76.39	74.25	
K ₂	77.47	81.60	75.42	76.56	
K ₃	77.18	88.10	75.25	76.26	
R	5.06	30.73	1.14	2.31	

SEM micrographs of RM floating microsphere cross-sections are shown in Fig. 1B. As it is difficult to study the internal structure of small microspheres, larger microspheres were employed in this work for investigation. They show a hollow/porous formation, with, in addition, a few pinholes visible on the internal surface of the hollow spaces.

2.3. Drug loading, entrapment efficiency and buoyancy

Three batches of microspheres were prepared based on the optimized formulation, according to the method given above; the results indicated high quality of the floating microspheres with DL and EE being $9.31 \pm 0.31\%$ and $89.55 \pm 1.65\%$, respectively. Furthermore, the floating microspheres showed a good floating ability, without buoyancy lag time, and remained floating for 12 h, while the percentage of floating microspheres was $91.45 \pm 1.62\%$. The floating properties of hollow/porous microspheres may be attributed to their low bulk density and porosity; implying that they will have the propensity to exhibit an excellent buoyancy effect *in vivo*.

2.4. In vitro drug release

Comparative *in vitro* drug release from various floating microsphere formulations in the orthogonal design is shown in Fig. 2. From the results, it is clear that the concentration of EC had an important influence on release from the microspheres. When the EC loading was 6%, the drug release profiles (formulations 1–3)

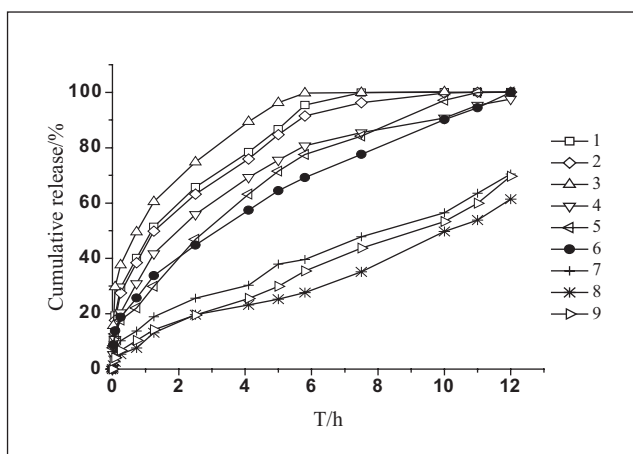


Fig. 2: *In vitro* drug release of RM floating microspheres of formulations 1 to 9

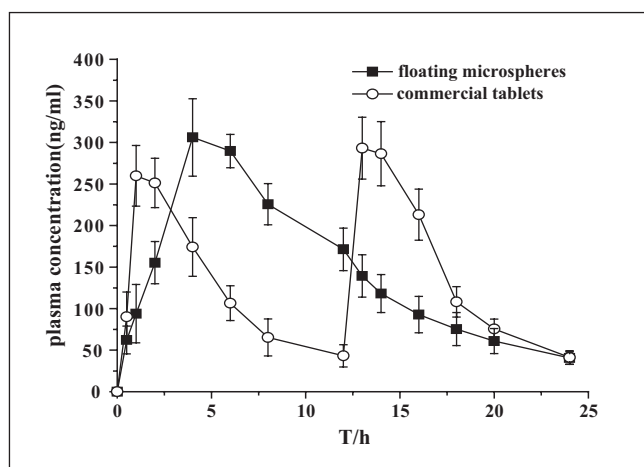


Fig. 3: Plasma concentration of RM from commercial tablets (4 mg/tablet) given at 0 h, 12 h compared to a single 8-mg drug dose given in floating microspheres. (Mean \pm S.D., $n = 6$)

exhibited a burst drug release in the initial stage. For formulations 4, 5 and 6 (10% EC), drug release was 76.24–90.60% within 8 h and 91.02–101.78% in 12 hours. As the concentration of EC was increased to 14% (formulations 7–9), initial drug release as well as drug release at later times was decreased, 10.56–21.42% drug release in 2 h, 28.64–40.39% in 6 h and 61.41–70.49% in 12 h.

2.5. Pharmacokinetic analysis

The limit of detection for RM was found to be $10 \text{ ng}\cdot\text{ml}^{-1}$ under the present conditions. The assay was linear in the range $29\text{--}725 \text{ ng}\cdot\text{ml}^{-1}$. The mean absolute recovery of RM in plasma was $82.18 \pm 1.35\%$, and the mean relative recovery was $103.01 \pm 3.62\%$. Intra- and inter-day accuracy was within the acceptance range of 90–110%. Intra- and inter-day precisions were $<3\%$, which was within the acceptance range.

RM plasma concentration profiles after administration of a single oral dose of 8-mg in sustained-release floating microspheres or two 4-mg commercial tablets are shown in Fig. 3, and the pharmacokinetic parameters are listed in Table 3.

A rapid increase in plasma concentration of RM resulted after administration of the 4-mg reference tablets, with C_{max} ($265.01 \pm 30.10 \text{ ng}\cdot\text{ml}^{-1}$) occurring at approximately 1.5 h, whereas C_{max} after administration of 8-mg floating microspheres ($312.36 \pm 38.34 \text{ ng}\cdot\text{ml}^{-1}$) occurred at 4.5 h. The T_{max} of RM after administration of the floating microspheres was significantly delayed ($P < 0.05$) compared with that after administration of the commercial tablets, indicating the sustained-release character of the floating preparation. The bioavailability of the floating microspheres relative to the ref-

erence tablets was $106.81 \pm 5.45\%$. This demonstrated that the bioavailability of RM was not reduced by its incorporation in a sustained-release floating microsphere system.

Pharmacokinetic studies of the sustained-release RM floating microsphere formulation have not previously been reported. The usual dosage regimen for RM commercial tablets in clinical practice is one tablet twice a day. In this study, a different dosage regimen was adopted, i.e., 8-mg once a day for the sustained-release floating microspheres and a 4-mg tablet twice daily for the conventional tablets, so that possible accumulation and fluctuation after administration could be correctly compared. In general, fluctuation changes according to the dosage frequency for the same medication at the same dose. It is obvious that significant differences exist between the two treatments as regards the fluctuation found in our study. The release of RM from the floating microspheres was sufficiently slow to allow once-daily administration, decreasing the fluctuation of RM plasma concentration. This is of clinical value, because a more frequent dosage regimen is often associated with poor compliance.

In conclusion, the *in vivo* study suggested that the sustained release microsphere system could reduce the number of daily administrations without increasing the fluctuation of plasma concentrations and decreasing the bioavailability of RM. Therefore, once-daily RM floating microspheres might be an alternative to the currently available 4-mg RM conventional tablet.

3. Experimental

3.1. Materials

Rosiglitazone maleate (99.1% purity) was provided by Chengdu Yuyang High-Tech Developing Co. Ltd. (China). Rosiglitazone maleate tablets were bought from GlaxoSmithKline Co. Ltd. (China). α -Asarone was provided by the National Institute for the Control of Pharmaceutical and Biological Products. Ethyl cellulose (EC, 20 cps) was provided by Shanghai Colorcon Co. Ltd. Poloxamer 188 and octadecyl alcohol were provided by Shang Hai Xie Tai Chemical Industry Co. Ltd. Acetonitrile (HPLC grade) was obtained from Tianjin Kermel Chemical Reagent Co. Ltd. All other reagents were of analytical grade.

3.2. Preparation of sustained-release floating microspheres

The microspheres were prepared by the emulsion-solvent diffusion method. Briefly, the drug, ethyl cellulose and octadecyl alcohol were co-dissolved in a mixture of ethyl acetate and dichloromethane (7:3, v/v) at room temperature. Poloxamer 188 was dispersed in water saturated with ethyl acetate as an aqueous phase. This drug-polymer solution was slowly added to 100 ml of aqueous phase with gentle stirring at 100 rpm. The resultant emulsion was added dropwise with 100 ml phosphate buffer (pH 6.8) under agitation for 30 min. As the organic solvent evaporated, the droplets gradually solidified and formed microspheres. The microspheres were collected, washed with distilled water and oven-dried (40°C).

3.3. Scanning electron microscope analysis

Morphological examination of the microspheres was with a KYKY 2800b scanning electron microscope (KYKY Technology Development Ltd. Beijing, China). Prior to examination, the samples were gold sputter-coated to render them electrically conductive.

3.4. Determination of drug loading and encapsulation efficiency

The samples were assayed using a validated HPLC method. The HPLC system consisted of a P3000A pump (Beijing Chuang Xin Tong Heng Science and Technology Co. Ltd.), and a fluorescence detector, using an excitation wavelength of 245 nm and an emission wavelength of 392 nm. The separation was performed on a Venusil XBP C18 column ($5 \mu\text{m}$, $250 \times 4.6 \text{ mm}$) and the injection volume was $20 \mu\text{l}$. The mobile phase was composed of phosphate buffer (pH 6.8)-acetonitrile-methanol (60:25:15, v/v/v). The flow rate was $1.0 \text{ ml}\cdot\text{min}^{-1}$.

DL and EE of the microspheres were determined using the HPLC method described above, and calculated using Eqs. (1) and (2), respectively. For each batch of microspheres, a quantity of 1.0 g was taken and ground to fine powder. About 150 mg powder was then accurately weighed and added to

Table 3: Pharmacokinetic parameters of RM commercial tablets and floating microspheres (mean \pm S.D., $n = 6$)

Parameters	Reference tablets	Floating microspheres
K_e/h^{-1}	0.16 ± 0.01	0.10 ± 0.02
$T_{1/2}/\text{h}$	4.34 ± 0.30	7.01 ± 1.33
$\text{AUC}_{0-\infty}/\text{ng}\cdot\text{h}\cdot\text{ml}^{-1}$	3723.93 ± 693.42	3951.02 ± 557.25
$C_{\text{max}}/\text{ng}\cdot\text{ml}^{-1}$	265.01 ± 30.10 307.01 ± 33.93	312.36 ± 38.34
T_{max}/h	1.33 ± 0.52 13.50 ± 0.55	4.67 ± 1.03
Fr/%		106.81 ± 5.45

a 100 ml volumetric flask containing 70 ml of mobile phase. After 30 min of ultrasonic extraction, the suspension was diluted with mobile phase to 100 ml and then filtered through a 0.45 μm membrane. The filtered solution was injected for analysis. All samples were analyzed in triplicate.

$$\text{DL}(\%) = \frac{W_D}{W_T} \times 100 \quad (1)$$

W_D : the weight of the drug loaded in the microspheres; W_T : the total weight of the microspheres.

$$\text{EE}(\%) = \frac{W_A}{W_T} \times 100 \quad (2)$$

W_A : actual drug content; W_T : theoretical drug content.

3.5. Buoyancy

Microspheres (100 mg) were dispersed in a solution composed of 500 ml simulated gastric fluid (SGF, pH 1.2, without enzymes). The mixture was stirred with a paddle at 100 rpm and $37 \pm 0.5^\circ\text{C}$. After 12 h, the microspheres that floated over the surface of medium and those that settled to the bottom were recovered separately. Both particle types were dried at 40°C overnight. Each fraction of the microspheres was weighed and buoyancy was represented by the following Eq. (3):

$$\text{Buoyancy}(\%) = \frac{Q_f}{Q_f + Q_s} \times 100 \quad (3)$$

Q_f : weight of floating microspheres; Q_s : weight of settled microspheres.

3.6. *In vitro* release studies

Studies of drug release from the microspheres were performed in triplicate using the Chinese Pharmacopoeia 2005 apparatus type II (paddle method). The microspheres were incubated in 900 ml of simulated gastric fluid (SGF, pH 1.2, without enzymes) and maintained at $37 \pm 0.5^\circ\text{C}$ with a rotation speed of 50 rpm. The quantity of microspheres was chosen to be equivalent to 4 mg drug. At different time intervals, 3 ml of the solution was withdrawn and replaced by 3 ml of fresh dissolution medium. Samples were assayed using the HPLC method described above.

3.7. *In vivo* evaluation of floating microspheres

The bioavailability study was in accordance with GCP/GLP standards. The protocol was approved by an ethics committee on bioavailability studies. Six healthy male volunteers, age 22.2 ± 0.8 years, weight 64.2 ± 3.9 kg, participated in the study after providing written informed consent. The volunteers were judged to be healthy and were not receiving any medication during the study period. Volunteers were given information on the drug and nature of the study in advance of the trial. The study was conducted according to a randomized, two-period crossover design, and the washout period was 1 week between treatments of the study. Six volunteers fasted overnight (10 h) were randomly selected to receive a single dose of floating microspheres equivalent to 8 mg RM or 2 reference tablets (4 mg/tablet, one administered at 0 h and the other administered at 12 h in the study period) with 250 ml of water. Blood samples of 5 ml were collected from the forearm vein into heparinized centrifuge tubes at 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 13.0, 14.0, 16.0, 18.0, 20.0 and 24.0 h exactly. After centrifugation, the plasma was pipetted into polypropylene tubes, frozen immediately and stored at -20°C until analysis.

The drug levels in the plasma samples were determined using the HPLC method described above. 300 μl of plasma from the stored samples was added to centrifuge tubes containing 3 ml acetic ether and 100 μl α -asarone ($5.0 \mu\text{g}\cdot\text{ml}^{-1}$) as the internal standard. The samples were shaken for 5 min followed by centrifugation ($2325 \times g$), and the supernatant solution was transferred to a new tube and evaporated under nitrogen in a 40°C water bath. The residue was dissolved with 100 μl mobile phase and then centrifuged, and 20 μl of supernatant was injected for analysis.

3.8. Pharmacokinetic analysis

Pharmacokinetic parameters were established using established non-compartmental methods. C_{max} and t_{max} were obtained directly from these curves. AUC_{0-t} was calculated using the trapezoidal method. $\text{AUC}_{0-\infty}$ was calculated by: $\text{AUC}_{0-\infty} = \text{AUC}_{0-t} + C_t/K_e$, where C_t is the plasma RM concentration observed at 24 h and K_e the apparent RM elimination rate constant obtained from the terminal slope of the individual plasma concentration-time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The biological half life ($t_{1/2}$) was calculated by: $t_{1/2} = 0.693/K_e$.

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