Formulation Optimization of Gastro-Retention Tablets of Paeonol and Efficacy in Treatment of Experimental Gastric Ulcer

Xitong Zhang, Yue Zhang, Han Han, Jun Yang, Benliang Xu, Bing Wang and Tong Zhang

"Experiment Center for Teaching and Learning, Shanghai University of Traditional Chinese Medicine; No. 1200, Cailun Road, Pudong New District, Shanghai 201203, China; Shanghai Xiangshan Hospital of Traditional Chinese Medicine; No. 352, Middle Fuxing Road, Huangpu District, Shanghai 200020, China; and School of Pharmacy, Shanghai University of Traditional Chinese Medicine; No. 1200, Cailun Road, Pudong New District, Shanghai 201203, China.

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This study aims to develop a gastroretentive sustained-release drug delivery system of paeonol using floating properties and to investigate its therapeutic effects in rat models. The gastric retention tablets of paeonol (GRT-Ps) were prepared by a direct compression method, and the Box–Behnken design was used to optimize its formulation. The optimized formulation containing 15% NaHCO3 and a 2:1 ratio of paeonol and HPMC-K4M floated within 1 min and remained afloat for more than 8 h in the simulated gastric fluid (200 mL, pH=1.2) and simultaneously showed the desired sustained drug release. Moreover, small tablets (3 mm) were prepared according to the same formulation and the process technology of big tablets (8 mm). A similar drug release behavior was observed between two kinds of tablets (f2=52), and then the evaluations of efficacy and retention capacity in vivo were conducted with small tablets. In vivo retention studies showed that the Tmax (2 h) of GRT-P in rat stomachs was significantly extended compared with the Tmax (0.5 h) of normal reference preparation. Compared with the model group, low and high doses of GRT-P could significantly inhibit the increase of malondialdehyde (MDA) in serum. Studies showed that the higher MDA content in inflammation tissue increases the inflammatory response. The ulcer inhibition rates of GRT-P in the high-dose group were 59.0 and 64.1% in the ranitidine group. Results indicated that GRT-Ps had the potential for a sustained drug release and an enhanced gastric residence time with relatively high drug concentrations in the tissue distribution.

Key words paeonol; gastric retention tablet; sustained release; gastric ulcer; efficacy

Paeonol, also called peony phenol, is the active component of a traditional Chinese medicine, namely, moutan cortex (Paeonia suffruticosa Andrews, Ranunculaceae). Paeonol has been extensively studied for its anti-inflammatory, antioxidant, anti-atherosclerosis, anti-diabetic, and anti-mutagenic effects. However, paeonol has significant growth-inhibitory and apoptosis-inducing effects in gastric cancer cells in vitro and in vivo. Moreover, studies have shown that paeonol prevents inflammatory diseases of the alimentary canal, such as irritable gastric ulcer, with minimal side effects. However, studies on the ethanol-induced gastric ulcer effect of paeonol are lacking. Currently, the listed paeonol dosages are administered via ordinary oral tablets and injection. However, paeonol has poor water solubility, easy oxidation, and a short biological half-life. The paeonol products are unable to prolong effective drug concentration in blood to influence the oral bioavailability of a drug. Therefore, the present study, which is based on the properties of paeonol, has been designed to develop gastric retention tablets using modern technology to achieve prolonged presence in the stomach, thereby improving treatment of gastric ulcer. This approach has a profound clinical significance in developing a novel sustained-release delivery system with good local treatment for improving the therapeutic effect.

Gastric retention preparation can extend drug release duration, increase local treatment concentration, and improve drug absorption and bioavailability. Most oral sustained-control release drug delivery systems effectively regulate drug release into the system; however, drug bioavailability is low because of low drug concentration in local tissue. Therefore, gastric retention agents are significant in treating stomach diseases with the advantages of improving drug efficacy and reducing drug toxicity.

Preliminary experiments showed that paeonol has certain inhibitory effects for Helicobacter pylori and gastric ulcer. We aimed to develop a sustained-release oral dosage of paeonol to increase the partial blood–drug concentration in the stomach. The sustained-release system can achieve effective therapeutic drug concentrations in the systemic circulation over an extended period. In this study, we selected the floating system to increase gastric residence time and to sustain drug release for developing gastric retention tablets of paeonol (GRT-P). We designed the formulation of gastric retention and sustained release for 8 h by considering gastric emptying time, gastric retention factors, and dietary habits.

Experimental
Preparation of GRT-P

Paeonol is volatile with low melting points, thereby causing difficulty in using the wet legal system grain. We selected direct powder compression considering the high influence of the drug product surface properties on drug dissolution. Drug directly released from powder after tablet disintegration, which has a considerable dispersion, can accelerate dissolution and can improve rela-

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tive bioavailability. Moreover, direct powder compression is simple and does not require granulating and drying, thereby ensuring a stable quality of the finished product. The selection of appropriate accessories for direct powder compression is important. Auxiliary materials should have good liquidity, compressibility, suitable bulk density, and large pharmaceutical accommodation. Therefore, this study selected hydrophilic gel skeleton materials, hydroxypropyl methyl cellulose, foaming agent sodium bicarbonate, flow aid powdery silica gel, filler lactose, and microcrystalline cellulose as tablet accessories. All materials and accessories passed through sieve no. 80 separately. The powder blend was then compressed into tablets using an 8 mm flat-face round tool on a single-punch tablet machine (Cadmach, Ahmedabad, India). The tablet contained 80 mg paeonol and maintained 200 mg tablet weight. The compression force was adjusted to obtain tablets with hardness in a range of 4–5 kg/cm². We first mixed the active pharmaceutical ingredients and micro-powder silica gel to ensure paeonol content uniformity in the tablets and then blended them again with lactose and microcrystalline with good liquidity; finally, we blended all the ingredients.

Statistical Design and Analysis Recently, the response surface method experimental design has been widely used in screening for new drug delivery systems to ensure prescription and process optimization. The Box–Behnken design (BBD), a response surface method, aims to establish the relative importance of two or more factors; this design indicates the interaction between various factors that influence the magnitude of the response. Short cycles and high precision can establish a continuous variable surface model by evaluating the influencing factors and their interactions in the process through a method with advantages of few tests to determine the most efficient level and to decrease the experimental group number, which can save manpower and material resources. In this study, according to single-factor experiments, three main factors (contents of HPMC-K4M, NaHCO₃, and Avicel) were selected, and their proper ranges were determined. The results of the single factor study reduced the range of HPMC-K4M to between 15 and 30%, NaHCO₃ between 15 and 22.5%, and Avicel between 5 and 12.5%. A 17-run BBD, including 12 factorial and 5 axial points, was applied to determine the optimal prescription. Three variables were presented, namely, X₁ (the amount of HPMC-K4M), X₂ (the amount of NaHCO₃), and X₃ (the amount of Avicel), along with three levels coded 1, 0, and −1 for high, intermediate, and low, respectively. The response values were Y₁: drug accumulative release at 2 h in vitro (%) and Y₂: drug accumulative release at 8 h in vitro (%). The factors and the response surface analysis are listed in Table 1.

Morphological Study on GRT-P The release behavior of paeonol from the matrix tablets was studied in vitro. A scanning electron microscopy (SEM) was used to inspect the porosity and morphology of the drug-released tablets after they swelled in the release medium. The effects of the release enhancer and surfactant on the release of paeonol were also investigated. The release mechanism was preliminarily determined through the morphological observation and change of structure.

In Vitro Release Rule and Mechanism A relevant mathematical model can be used in fitting the drug release curve of preparation in vitro. In vitro average cumulative release rate and time of GRT-Ps were fitted according to the different mathematical models; three release equations were fitted, namely, zero order, first order, and Higuchi equations, which were used to describe the mechanism of drug release of the tablets. The data obtained from in vitro drug release studies were used to calculate the correlation coefficient (r) between the cumulative amount of drug released and time. The best-fitting model was considered concerning the one that had a maximum r value. The three models are presented as follows:

Zero-order release equation: \[ M_t / M_\infty = K_t \]
First-order release equation: \[ \ln(1 - M_t / M_\infty) = -k_t t \]
Higuchi release equation: \[ M_t / M_\infty = K_t t^{1/2} \]

In addition, Korsmeyer–Peppas equation \( (M_t / M_\infty = K_t t^n) \) was used to determine the release mechanism of paeonol from tablets. If 0.45 < n < 0.89, then the drug release mechanism confirms Fick's diffusion law of the synergistic effect of skeleton dissolution and diffusion.

In Vitro Floating Ability Study The floating behavior of the tablets was visually determined in triplicate according to the floating lag-time method described by ref. 27. A tablet was placed briefly in a glass beaker containing 100 mL of simulated gastric fluid and was maintained in a water bath at 37±0.5°C. The floating lag time (the time that GRT-P floated from the bottom to the surface of the liquid) and the total floating duration (from the GRT-P on float to the tablet dissolution disappearance or sinking time) were recorded. The design goal of floating lag time in vitro was less than 3 min, and the tablets could maintain a floating state for more than 8 h.

In Vitro Drug Release Behavior Drug release studies on the prepared GRT-Ps were performed in triplicate consistent with General Requirements 0931, Volume IV of the Pharmacopoeia of the People’s Republic of China 2015 edition. The tablets were placed in a 900 mL simulated gastric fluid solution containing 0.25% sodium dodecyl sulfate (SDS) at 37±0.5°C water bath with paddles rotated at a speed of 100 rpm. Samples (5 mL) were withdrawn from the dissolution apparatus in each vessel after 2, 4, 6, and 8 h and were filtered through a cellulose acetate membrane (0.45 μm). The drug content was determined through the HPLC spectrophotometric method at 274 nm. In each withdrawal, 5 mL of fresh medium was replaced into the dissolution flask.

Assessment of Stomach Retention Effect Male Sprague-Dawley (SD) rats fasted for 12 h after receiving water and were mildly anesthetized with ether. The 3 mm GRT-Ps was administered intragastrically compared with the reference preparation of homemade conventional tablets. After 0.5, 2, 4, 6, and 8 h treatments, the five rats were executed at each time point. Stomach tissue was collected through dissection by immediately cutting open along the great curvature of the stomach. Then, the stomach tissue was rinsed with 5 mL physiological saline. If the whole tablets were visible, then
they were taken out of the stomach. The flashed gastric contents were cryopreserved in ~80°C. After the gastric tissue was blotted with filter paper, precisely weighed, and cut, the corresponding volume of physiological saline was added to the tissue samples (1 g stomach tissue: 3 mL saline volume)\(^{32,33}\) with the homogenate instrument to shear and homogenate, and then the serum was set aside for ~80°C cryopreservation. Two sets of samples with the lyophilizer were dried, and the dry solid samples were dissolved in 2 mL methanol. We filtered the supernatant obtained after centrifugation by 0.22µm filter membrane filtration and determined its content through the HPLC method.

Establishment of Gastric Ulcer Models A total of 40 SD male rats weighing approximately 250–270 g were randomly divided into five groups, namely, blank, model, positive control ranitidine hydrochloride, low-dose, and high-dose groups, according to weight. The drug doses were as follows: low-dose group: 24 mg kg\(^{-1}\), high-dose group: 48 mg kg\(^{-1}\), and ranitidine-positive control group: 25 mg kg\(^{-1}\). After fasting with water for 12 h before models were established, all groups except the blank group were given 0.5 mL 100 g\(^{-1}\) of anhydrous ethanol by lavaging mode. After establishing the model for 2 h, each dose group was given the drug according to the aforementioned dosages by lavage, and the normal control and model groups were given 1 d\(^{-1}\) saline for 6 d. The rat gastric volume was 1 mL 100 g\(^{-1}\). Then, 2 h after the last delivery, the rat abdomen was split to remove the abdominal aortic blood, and then pylori and cardia were ligated to take the whole stomach. The mice were cut along the greater curvature of the stomach, which was then washed with physiological saline. According to the standard of Okabe ulcer index, the length and width of ulcer surface were measured with a vernier caliper, calculated ulcer index, and ulcer inhibition rate; and then a part of ulcer gastric tissue was sheared to determinate the content of hexosamine in the supernatant of tissue homogenate; furthermore, the residual gastric tissue was fixed in 4% formaldehyde to prepare pathological sections for further observation of ulcer formation and healing.\(^{34–36}\)

Table 2. Corresponding Surface Analysis Method and Result Analysis

| Run order | HPMC-K4M (mg) | NaHCO3 (mg) | Avicel PH-102 (mg) | 2h drug accumulative release (%) | 8h drug accumulative release (%) |
|-----------|---------------|-------------|-------------------|-------------------------------|-------------------------------|
| 1         | 42.5          | 37.5        | 17.5              | 39.6                          | 67                            |
| 2         | 55            | 45          | 17.5              | 29                            | 59.9                          |
| 3         | 42.5          | 30          | 25                | 47.6                          | 80.3                          |
| 4         | 42.5          | 37.5        | 25                | 38.5                          | 64.9                          |
| 5         | 55            | 37.5        | 25                | 30.4                          | 62.1                          |
| 6         | 42.5          | 45          | 25                | 84.8                          | 90.4                          |
| 7         | 42.5          | 45          | 10                | 73.5                          | 91.2                          |
| 8         | 55            | 37.5        | 10                | 21.2                          | 50.5                          |
| 9         | 30            | 37.5        | 25                | 87.6                          | 94.8                          |
| 10        | 42.5          | 30          | 25                | 54.7                          | 88.5                          |
| 11        | 30            | 45          | 17.5              | 95.2                          | 96.3                          |
| 12        | 30            | 30          | 17.5              | 50.1                          | 82.5                          |
| 13        | 30            | 37.5        | 10                | 66.7                          | 73.2                          |
| 14        | 55            | 30          | 17.5              | 23.6                          | 58                            |
| 15        | 42.5          | 37.5        | 17.5              | 24.3                          | 57.5                          |
| 16        | 42.5          | 37.5        | 17.5              | 45.6                          | 74.1                          |
| 17        | 42.5          | 37.5        | 17.5              | 38.3                          | 72.3                          |

The abovementioned animals were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). This study was approved by the Ethical Committee of the Laboratory Animal Center, Shanghai University of Traditional Chinese Medicine. The protocol approval number is SYXK (Shanghai) 2014-0008, and the date of approval is June 27, 2014.

Statistical Analysis All the data were presented as mean±standard deviation (S.D.) or mean. Multiple comparisons of means (ANOVA) were used to substantiate statistical differences between groups, and Student’s t-test was used to compare two samples. The significance was tested at the 0.05 level of probability (p). Data analysis was performed with SPSS software package (version 19.0, IBM, NY, U.S.A.) or Design-Expert software (version 8.0.6, Stat-Ease Inc., MN, U.S.A.).

Results and Discussion

Optimal Prescription Screening Results The BBD method of the central composite design establishes the relative importance of two or more factors and indicates whether or not the interaction occurs between factors, thereby showing the factor that affects the magnitude of the response.\(^{20–22}\) We used Design-Expert software (version 8.0.6, Stat-Ease Inc., MN, U.S.A.) to determine multiple regression fittings of the data in Table 2. Several statistical parameters were estimated, including p value and multiple correlation coefficients (r) provided by Design-Expert statistical software (version 8.0.6).

The results indicated that these full quadratic models were significant for all response values.

The model equation for the tablets in in vitro floating percentage at 2 h (%) is expressed as follows:

\[
Y_1 = 37.26 - 24.18A + 13.06B + 6.06C - 9.43AB -2.93AC + 1.05BC - 0.98A^2 + 12.69B^2 + 15.2C^2
\]

The model equation for the tablets in in vitro floating percentage at 8 h (%) is expressed as follows:

\[
Y_2 = 67.16 - 14.54A + 3.56B + 5.08C - 2.98AB - 2.5AC - 2.25BC - 5.22A^2 + 12.23B^2 + 8.21C^2
\]
The $r$ values of the two models were 0.9834 and 0.9605. The $p$ values for the lack-of-fit tests were 0.8722 and 0.6095, indicating that the full quadratic model adequately fits the response surface.

Based on the aforementioned fitting mathematical model, we depicted the corresponding contour map and independent variable on the dependent variable of the three-dimensional (3D) surface figure by using Design-Expert (version 8.0.6) software. The results are illustrated in Figs. 1 and 2. Based on the numerical optimization and our design goals, the desirable range of responses were $20% < Y_1 < 50%$ and $Y_2 > 90%$. According to the results of the software analysis, the overall model reached a significant level ($p<0.05$), indicating that the fitting quadratic equation model was significant. One of the factors is fixed at the center value, whereas the 3D maps and contour lines of $Y_1$ and $Y_2$ to other two factors are depicted respectively. Optimal formulations using $X_1=40$, $X_2=30$, and $X_3=24$ mg were prepared using the aforementioned method. The actual values and predicted responses generated by the mathematical model are presented in Table 3.

**Characterization of Tablets in SEM** When sodium bicarbonate mixes with gastric juices, carbon dioxide escapes from the film, and the tablet surface forms holes (Fig. 3), indicating that sodium bicarbonate plays a dual role as a pore forming and a bleaching agent. Simultaneously, the powder material hydroxypropyl methyl cellulose, which has a minimal density, swells after a certain period in the release medium to form a gel skeleton (Fig. 4) and begins to capture the carbon dioxide-produced holes, thereby widening the film; some drug release channels are formed by the gel skeleton combining with the aforementioned holes, and the swelling layer continues to be dissolved simultaneously. The floating time is

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Fig. 1. Contour and Surface Plots of 2 h Drug Release
Fig. 2. Contour and Surface Plots of 8h Drug Release

Fig. 3. Surface SEM Images of GRT-P (A: Before Swelling, B: After Swelling)

Fig. 4. Cross-Section SEM Images of GRT-P (A: Before Swelling, B: After Swelling)
extended when the drug release channel increases gradually, and the preparations are released in a slow manner. In this dynamic process, the tablets can continue to float in the release medium because the density of GRT-P core is smaller than the release medium.

**In Vitro Release Properties and Mechanisms** The Higuchi equation of the correlation coefficient $r^2=0.9985$ is higher than the other $r$ values; therefore, the optimal fitting model of the release law for GRT-P is the Higuchi equation, which indicates the good slow-release effect of the gastric retention tablets (Table 4).

Peppas equation is $\lg M_t/M_\infty=0.4704 \lg t+1.5341$, $n=0.4704$. Hence, the releasing mechanism of paenol is a synergistic effect of skeleton dissolution and diffusion.

**In Vitro Floating Ability Study** The experimental results show that the property *in vitro* floating of GRT-P has achieved the desired objective, with the short bleaching time within 3 min and maintaining floating *in vitro* for more than 8 h.

**In Vivo Gastric Retention Study** The GRT-P and the reference preparations of paenol (RP-P) after distribution in rat stomach tissue are illustrated in Fig. 5; the gastric contents are depicted in Fig. 6. The experimental results show that the distributions of GRT-P in the gastric contents and tissue of rats are larger than the RP-P, with the $T_{\text{max}}$ of 2 and 0.5 h, respectively; then, the drug–time curve of GRT-P has shown the obviously sustained-release effect of extending up to 8 h compared with RP-P. The area under curve (AUC) data also have the same trend in the gastric contents and tissue of rats, with the GRT-P having a higher gastric intake content and more gastric tissue distribution than RP-P; this result indicates that the GRT-P can improve gastric treatment concentration and enhance the treatment efficiency for gastric diseases.

**Preliminary Results of Pharmacodynamic Gastric Ulcer** Compared with the normal control group, the model group showed a significant increase in the malondialdehyde (MDA) content. Compared with the model group with $p<0.01$, the

### Table 3. Optimal Prescription of Actual Value Compared with Predicted Value

|                | $Y_1$ Drug accumulative release at 2 h (%) | $Y_2$ Drug accumulative release at 8 h (%) |
|----------------|------------------------------------------|------------------------------------------|
| Predicted value| 50.66                                    | 82.17                                    |
| Actual value   | 51.14                                    | 90.95                                    |
| Error rate     | 0.21                                     | 9.85                                     |

Error rate (%) = (Actual value − Forecast value)/Forecast value × 100%.

### Table 4. Fitting Results of Release Equation

| Model              | Equation         | $r$   |
|--------------------|------------------|-------|
| Zero order release | $Y=10.778X+13.630$ | 0.9519|
| First order release| $Y=0.1086X+3.6895$ | 0.9846|
| Higuchi release    | $Y=32.338X+0.5242$ | 0.9985|

Fig. 5. Stomach Tissue Medication in Curve Comparison ($n=5$)

Fig. 6. Gastric Content Medication in Curve Comparison ($n=5$)
low- and high-dose groups demonstrated that the gastric floating tablets significantly inhibited the increase of MDA in the serum (Fig. 7).

Compared with the model group, the ulcer index of the high-dose and ranitidine groups of the GRT-P were significantly lower \((p<0.01)\); statistical significance was observed, and no differences were found in ulcer index between the two groups. The results are presented in Table 5.

Compared with the blank group, the model group showed significantly decreased hexosamine content at \(p<0.01\). However, differences were observed. The high-dose group had the highest hexosamine content, indicating that the gastric retention tablets increased the hexosamine of gastric tissue and had certain therapeutic effects in the ethanol-induced gastric ulcers of the rat (Fig. 8).

Ethanol administration resulted in a significant epithelial desquamation, hemorrhage, and inflammatory cell infiltration in the mucosa. In the GRT-P high-dose group, the epithelial desquamation was largely prevented. Moreover, a slight inflammatory cell infiltration and limited hemorrhagic regions were observed. The results showed that ethanol administration resulted in severe damage to parietal cells, and the GRT-P treatment prevented the damage (Fig. 9).

**Conclusion**

Gastroretentive drug delivery systems are important in the delivery of drugs to the upper part of the gastrointestinal tract. In this study, gastroretentive sustained-release paenol tablets

| Group          | Dose (mg/kg) | Ulcer index (points) | Ulcer inhibition rate (%) |
|----------------|--------------|----------------------|---------------------------|
| Blank group    | —            | —                    | —                         |
| Model group    | —            | 3.90±1.13            | —                         |
| Low dose       | 24           | 3.00±0.76            | 23.10                     |
| High dose      | 48           | 1.60±0.92*           | 59.00                     |
| Ranitidine group | 25          | 1.40±0.92*           | 64.10                     |

\(^*p<0.01\) versus model group.
were successfully prepared with the desired release rates and floating durations. The preparation of the tablet formulation was stable, controllable, high-quality, highly sensitive, and characterized by simple detection. GRT-P had certain inhibitory effects on the gastric ulcer, with relatively high concentrations of tissue distribution, and good sustained-release ability to improve the treatment of gastric local concentration, which achieved the goal of treating gastric disease. This dosage form is promising for further in vivo studies, which can be used to develop new paeonol dosage forms.

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Conflict of Interest The authors declare no conflict of interest.

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