Preparation and pharmacokinetics in beagle dogs of ganershu sustained-release pellets

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ABSTRACT

Background: The active ingredients of Ganershu compound recipe, which are effective for hepatitis treatment in liver protection and transaminase reduction. However, the active ingredients of Ganershu compound recipe are poor absorption, which conduct it has a low oral bioavailability. **Objective:** We prepared Ganershu sustained-release pellets (GSPs) by fluidized-bed on central composite design-response surface methodology and increase its bioavailability in beagle dogs. **Materials and Methods:** In this study, GSPs were successfully prepared. The Drug-loaded pellets and sustained-release coated were carried out in fluidized-bed machine. GSP was optimized for fitting release, roundness, and the overall desirability by central composite design-response surface methodology. **Results:** To optimize cumulative release profile, the outermost ethyl cellulose coating layer and the hydroxypropyl methyl cellulose (HPMC) swelling layer were employed, which were respectively given coating levels in terms of weight gain of 22% and 6%, the concentration of HPMC is 4.5% (g/ml). The pharmacokinetics of Ganershu normal pellets (GNPs) and GSP was studied in beagle dogs after oral administration. The naringenin as an index, the area under the curve$_{0-\infty}$ of naringenin in GSP was 1.38 times greater than that of GNP. Meanwhile, *T*max of GSP was prolonged for about 74%. **Conclusion:** This study can clearly indicate that we enhanced the oral bioavailability of Ganershu by preparing the GSP, which had the sustained dissolution and improved the potential of it for clinical application.

Key words: Beagle dogs, fluidized-bed, Ganershu sustained-release pellets, naringenin, pharmacokinetics

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INTRODUCTION

Hepatitis is highly infectious, incurable, recrudesce and complex transmission way.[1] Then, there are few medicines, which are reliable efficacy, rapid onset, long action time and easy to use. In our previous study, we found that Ganershu compound recipe, which is consisted of *Sedum sarmentosum, FructusAurantii etc.*,[2,3] has the function of liver protection and transaminase reduction. Meanwhile According to our previous study, it was also shown that the active ingredients of Ganershu compound recipe which was prepared in our laboratory for hepatitis treatment are more effective.[4]

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However, the active ingredients of Ganershu compound recipe are poor absorption, which conduct it has a low oral bioavailability.

Sustained-release pellets, can be defined as small, free flowing, spherical particulates manufactured by the agglomeration of fine powers or granules of drug substances and excipients typically from about 0.5 mm to 1.5 mm. Multiple-unit sustained-release dosage forms of pellets are considered to have many therapeutic advantages in comparison with its multiple-unit dosage forms.[5] They can disperse in the gastrointestinal tract homogeneously thus maximize drug absorption, reduce peak plasma fluctuation, minimize the risk of local gastrointestinal tract irritation and dose dumping, decrease dosing frequency, increase patient compliance, improve the safety and efficacy of the active ingredient.[6-8]

However, few papers have been found about the influence of Ganershu sustained-release pellets (GSPs) on oral

absorption. Therefore, the present study is to prepare GSPs by fluidized-bed on central composite design-response surface methodology and evaluate its pharmacokinetics in beagle dogs.

MATERIALS AND METHODS

Materials and instruments

Talcum powder (Anhui Shanhe Pharmaceutic Adjuvant Co. Limited); ethyl cellulose (EC) Sieve (Nanjing Xiongshi Sieve Factory); electronic balance AG135 (Mettler Toledo in Swiss); air compressor (Xiameng East-Asia Mechinery, Co. Limited); knee type HH-S (Nanjing JingZheng Teaching Apparatus, Co. Limited); supercentrifuge (Shanghai AnTing Scientific Instrument Factory, TGL-16C); nitrogen blowing instrument HSC-12A (Tianjin HengAo Science, Co. Limited); electronic analytical balance FA-1104 (Shanghai Precise Scientific Instruments Co. Limited); magnetism msier 85-1 (Jintan RonghuaI nstruments, Co. Limited); multiskan spectrum (powerwave X430, Bio-Tek Co. Limited, USA); vortex mixing apparatus WH-3 (2306, Shanghai Huxi Analytical Instruments, Co. Limited); multifunction fluidized bed experimental machine WBF-2 (Chongqing Yingge Granulation and Coating Technology, Co. Limited); waters 2695 high-performance liquid chromatography (HPLC); 717 autosampler; water 2998 UV-Detector; Empower Chromatographic Work Station. The herbal medicines (*sarmentosum, bupleurum, phyllanthus, capillaris, paeonia lactiflora, atractylodes, Citrus aurantium, Citri reticulatae, gynostemma, licorice, Polygonatum sibiricum, malt, ligustrum, Corydalis*) of Ganershu compound recipewere verified by Jianwei Chen (College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, China).

Preparation and optimization of Ganershu sustained-release pellet

Preparation of Ganershu normal pellets

Three hundred gram commercially available blank pill core were weighted and placed in a fluidized bed side spray device. The coating dispersion was prepared according to the composition presented in Ganershu extract powder, and talc was used as an antitacking agent. The procedure to prepare the coating dispersion was as in the following parameters: Sprayed into the atomization pressure to 300 kPa, spray pump speed is 4 r/min, turntable height is 5 mm, inlet air temperature is 75°C, engine frequency is 30 Hz, turntable speed is 300 r/min, get the Ganershu normal pellets.

Preparation process of Ganershu sustained-release pellets

Reference to the principles of formulation of the coating controlled pellets,[9,10] Surelease was chosen as the membrane-former, turn the Ganershu normal pellets into release pellets depend on fluidized bed. Different from the chemical medicine, Chinese herbal compound had complex compositions, which need to design how to overall release the complex composition synchronized. Previous study[11] has shown that hydrophilic gel material as the first coating material, then coat the liposolubility material, which play the effect of synchronous release.

In this study, a bottom-spraying process coating of fluidized bed was taken to coat the swelling layer and the sustained-release layer. The main aspect to influence the effect of swelling layer and synchronous release is the concentration of the solution of hydroxypropyl methyl cellulose (HPMC) and the increase of weight, which viscosity is 6 MPa.s, choose the EC as the sustained release layer material which is an integrity solution contain aqueous dispersion of EC, oleinic acid as a stabilizing agent, dibutyl sebacate as plasticizer, ammonia water, and lightweight silicone sometimes. Solid content is 25%, diluted to 15% with water when use. Coating thickness is a key aspect to influence the drug release rate.

Taking bottom-spraying process coating of fluidized bed to prepare the GSP needs to choose the parameter of fluidized bed and the prescription composition.

Parameter optimization of the bottom-spraying process of fluidized bed

The process of the bottom-spraying process of fluidized bed is a little different from side spray, drum height, inlet air temperature, fans frequency, atomization pressure, inlet air temperature, fans frequency, atomization pressure, pump speed should be considered. According to the previous experiment, set the drum height 15 mm, pump speed 4 r/min well get a suitable condition. Inlet air temperature, fans frequency, atomization pressure play an important role in a film. After a single factor test with these three factor, the result is that fixed other conditions, when set the fans frequency 30 Hz, we get a well coating consequent because pellets in suspension flow state; when set the inlet air temperature 50°C, the mobility of pellets is well and is difficult to stick, that is because Sulease reflect a favorable membrane at a specified temperature. And then set the atomization pressure 250 kPa, the coating solution showed a small droplet state that could distribute at the surface of the pellets.

Optimization of the coating prescription

Investigated indexes

With reference to dissolution rate standards of chemical drug release of sustained-release preparation, the cumulative release rate at 1, 4, 8 h (expressed by Q_{1h}, Q_{4h},

Q_{8h}) and roundness of sustained-release pellets were also selected as investigated indexes.

The total flavonoids, total saponins and luteoloside in *sarmentosum* (monarch drug) were selected as determination indexes in cumulative release experiment of GSP. Adopted the method of weighting to set multi-index evaluations when referring to them, Mathematics Model were constructed with composite index of effective parts and monomer composition in experiment. Weighting coefficient of effective parts (A) was 0.8 and monomer composition was 0.2. The content of total flavonoids (A_1) total saponins (A_2) in effective parts are 21.6 mg/g and 33.7 mg/g. Then the optimized weighing coefficient of flavonoid and saponins depend on the ratio of their content, that is 21.6:33.7, the weighted coefficient of flavonoid was 0.39 and saponins was 0.61; The monomer composition mignonette weighting coefficient was 0.2. Integrated release rate (Q) was calculated using the following formulas:

$$Q = 0.8 \times (0.39A_1 + 0.61A_2) + 0.2 \times B \quad (1)^{[12]}$$

A_1, A_2 and B are average release rate of each component.

The cumulative release at 1, 4, and 8 h are integrated by multiple composition indexes. To simplify indexes and determine the effect of the sustained-release, match the release rate of three points into Y with release interval method. Calculation formula for Y values is:

$$Y = |Q_{1h} - 18| + |Q_{4h} - 50| + |Q_{8h} - 75| \quad (2)^{[13,14]}$$

Y was fitting calculated according to the sustained-release preparation standards that the sustained-release pellets cumulative release 18% at 1 h, 50% at 4 h, 75% at 8 h in actual calculation. The lower Y is the more accord with standards.

$$Q = 0.8 \times (0.39A_1 + 0.61A_2) + 0.2 \times B$$

The method determining the roundness of sustained-release pellets, which called planar marginal stability act is consistent with previously blank pellets.

In the design of experiment,[15,16] if the investigated index for a single index, its optimization can be judged based on results directly; but when the indexes for multiple targets, indexes may influence each other, such as: Beneficial to the rounding parameter may adversely affect the friability. We selected the comprehensive effect based on these indexed by referencing to the relevant studying to determine the optimal parameters. Overall therefore the introduction of "overall desirability" (OD) concept, each of the indexes are normalized between 0 and 1 of the OD, OD of each index to calculate the geometric mean, to get general comment of OD. The formula is as follows:

$$OD = (d_1 d_2 \ldots d_n)^{1/n} \quad (3)^{[17,18]}$$

n is number of indexes

For the factors which are in proportion (inverse) to values, the method--Hassan was adopted to evaluate "OD" d_{min} and d_{max}, the formula is as follows:

$$d_{\min} = (y_{\max} - y_i) / (y_{\max} - y_{\min}) \quad (4)$$

$$d_{\max} = (y_i - y_{\min}) / (y_{\max} - y_{\min}) \quad (5)$$

y_i is measured value of evaluation index, y_{min} is minimum of evaluation index, y_{max} is maximum of evaluation index.

The smaller roundness (Y_2) and fitting release (Y) are, the better result is. So d_{min} was employed to calculate d.

To sum up, the final investigated indexes are roundness (Y_2), fitting release(Y)and their OD.

Experimental design

Based on the pre-experiments, the prescription of GSP was optimized by central composite design-response surface methodology.[19-21] EC coating weight gain (X_1), the HPMC content of swelling layer (X_2) and weight gain percent (X_3), which had significant influence on cumulative release rate of GSP were selected as investigated factors, studied at five levels each [Table 1]. The detailed experimental design and the results were shown in Table 2.

The studies on pharmacokinetics of Ganershu sustained-release pellet and Ganershu normal pellet

Analytical methods

High-performance liquid chromatography was performed with Waters 2695 system, equipped with a binary solvent delivery system, auto-sampler, and a diode array detector. The chromatography was performed on Hedera ODS-2

Table 1: Values and levels of operating parameters

Independent variable	Levels				
	−1.732	−1	0	1	1.732
EC coating weight gain X_1/%	12	16.23	22	27.77	32
The HPMC content of swelling layer X_2/%	1	2.48	4.5	6.52	8
Weight gain percent of swelling layer X_3/%	2	3.69	6	8.31	10

EC: Ethyl cellulose; HPMC: Hydroxypropyl methyl cellulose

Table 2: Experimental design and the results (*n*=3)

Levels			Q_{1h}/%	Q_{4h}/%	Q_{8h}/%	*Y*	Roundness Y_2/°	OD
X_1	X_2	X_3						
16.23	2.48	3.69	24.13	53.24	77.31	11.68	23	0.119956877
27.77	2.48	3.69	21.15	50.31	75.98	4.44	21	0.627965881
16.23	6.52	3.69	16.23	52.49	80.03	9.29	22	0.325673235
27.77	6.52	3.69	20.05	52.41	78.98	8.44	20	0.516815071
16.23	2.48	8.31	22.33	47.65	76.97	8.65	22	0.356031299
27.77	2.48	8.31	18.67	50.14	74.56	1.25	21	0.740992801
16.23	6.52	8.31	22.89	47.36	76.14	8.67	23	0.2511091
27.77	6.52	8.31	15.45	45.64	71.52	10.39	23	0.187740684
12	4.5	6	23.86	53.12	78.59	12.57	24	0
32	4.5	6	15.65	45.78	70.31	11.26	19	0.325424913
22	1	6	20.48	52.14	77.13	6.75	21	0.531315233
22	8	6	17.23	48.12	73.26	4.39	20	0.727338867
22	4.5	2	19.36	52.63	76.82	5.81	22	0.4675397
22	4.5	10	17.12	48.25	73.67	3.96	21	0.646237791
22	4.5	6	17.89	50.03	75.31	0.45	20	0.885342787
22	4.5	6	18.1	49.92	75.02	0.2	20	0.894427191
22	4.5	6	18.03	50.13	75.13	0.29	20	0.891167474

OD: Overall desirability

column (4.6 × 200 mm, 5 μm). The mobile phase consisted of (A) aqueous solution contained 0.1% phosphoric and (B) acetonitrile. The HPLC elution condition was optimized as follows: 0-10 min (20-40%B), 10-30 min (40-80% B), 30-35 min (80-20% B). Column temperature was maintained at 30°C. The injection volume of standard and the sample was 30 μL. The flow rate was set at 1.0 ml/min. The detection wavelength was set at 288 nm.

Sample preparation

A volume of 200 μL of the plasma sample was transferred into a conical tube, 30 μL internal standard luteolin (0.00556 mg/mL) was added, then the mixture was stirred. A volume of 20 μL ascorbic acid (200 mg/mL) was added, and the mixture was agitated for 5 min and sequentially 80 uL HCL solution (10 moL/L) was added and eddied for 5 min; finally 1.5 mL diethyl ether was added, agitated and extracted for 5 min, and centrifuged for 5 min at 5000 r/min. Ether layer was transferred to another clean tube, and 100 uL acetonitrile water solution (48%) was added and dissolved with ultrasonic treatment after the diethyl ether evaporating, then at 15000 r/min the mixture was centrifuged for 10 min. 30 μL was injected into HPLC, and the chromatogram was recorded.

Validation of the high-performance liquid chromatography method

Specificity and selectivity

Blank plasma were prepared and tested for endogenous interference. Blank matrix sample spiked with naringenin and luteolin (the internal standard) were analyzed in order to assess potential interferences that may affect either the analyte or the internal standard.

Calibration curves

Stock solution of naringenin were prepared and diluted to appropriate concentrations for the construction of calibration curves. Six concentrations of the solution were analyzed in triplicates, the calibration curves were constructed by plotting peak area ratios of naringenin to the internal standard versus the concentrations of the naringenin.

Precision, accuracy and recovery

Precision and accuracy were chosen to determine the precision and accuracy of the HPLC method. Samples were extracted and analyzed as described in pretreatment of plasma samples and analytical methods. They were performed by triplicate extraction and analysis. Variations were expressed by the relative standard deviations (RSDs) and relative error %.

The recovery test was also used to evaluate the accuracy of the method. Accurate amounts of naringenin were added to blank plasma and then extracted and analyzed as described in pretreatment of plasma samples and analytical methods.

Oral bioavailability studies

In single-dose crossover study,[22] the Beagle dogs were randomly divided into two groups, fasting for 12 h, and take the blank plasma, then GSP and GSP (0.9 g/kg, equivalent to 536 g of crude drug, clinical adult daily dose 2 times) were administered by gastric perfusion, respectively. Take 5 ml of plasma at 0.083 h, 0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 3 h, 4 h, 5 h, 5.5 h, 6 h, 8 h, 12 h, and 24 h

after administration and centrifuge for 15 min at 3000 r/min in centrifugal tube containing heparin. The plasma was separated and treated according to "sample preparation".

Statistical analysis

All experiments were performed at least in triplicate. Data are presented as the mean ± standard deviation. The data were analyzed by Student's *t*-test. A two-tailed *t*-test (Microsoft Excel) was used to determine significant differences ($P < 0.05$) compared with the controls.

RESULTS AND DISCUSSION

Sustained-release pellets were divided into the matrix and coated controlled pellets according to the difference of formulation structure or drug release mechanism. Compared with the coated controlled pellets, matrix pellets possessed the advantage of the simple process, low cost, etc., Sustained-release matrix pellets were prepared by extrusion-spheronization mechanism previously, while, we found that it could not be extruded because of high viscosity and high hygroscopic of the powder, so finally gave up the further research of sustained-release matrix pellets, coated controlled release pellets were the emphasis in this paper.

The optimization of the coating prescription

Experimental design and the results of the optimization of the coating prescription

The experimental results [Table 2] were analyzed by Statistica 7.0 software. *Y* value, roundness and OD value were taken as the response variable; multi-linear regression and bivariate polynomial fitting were generated for all the response variables. We evaluated the fitting of the model according to *r*, The larger the value, the better the fitting. The equations of two models are represented as follows:

Multiple linear regression equation:

$$Y = 82.24181 - 5.40354\,X_1 - 3.79351\,X_2 - 3.85041\,X_3 \quad (r = 0.7868)$$

$$Y2 = 38.60025 - 1.00701\,X_1 - 0.69048\,X_2 - 1.38958\,X_3 \quad (r = 0.81449)$$

$$OD = -4.55120 + 0.35460\,X_1 + 0.20863\,X_2 + 0.28331\,X_3 \quad (r = 0.89510)$$

Bivariate polynomial equation:

$$Y = 107.1536 - 6.2877\,X_1 - 8.6665\,X_2 - 5.2578\,X_3 + 0.1183\,X_1^2 + 0.4477\,X_2^2 + 0.3000\,X_3^2 + 0.1663\,X_1X_2 + 0.0226\,X_1X_3 + 0.2023\,X_2X_3 \quad (r = 0.92213)$$

$$Y2 = 46.99221 - 1.22408\,X_1 - 1.73008\,X_2 - 2.61128\,X_3 + 0.01904\,X_1^2 + 0.07384\,X_2^2 + 0.11907\,X_3^2 + 0.01072\,X_1X_2 + 0.02813\,X_1X_3 + 0.13394\,X_2X_3 \quad (r = 0.0.89563)$$

$$OD = -6.37548 + 0.41278\,X_1 + 0.51017\,X_2 + 0.45195\,X_3 - 0.00761\,X_1^2 - 0.02406\,X_2^2 - 0.02295\,X_3^2 - 0.00821\,X_1X_2 - 0.00354\,X_1X_3 - 0.02016\,X_2X_3 \quad (r = 0.96513)$$

From the above equation, correlation coefficients of bivariate polynomial fitting were significantly better than the multiple linear regression equation in all indexes.

Three-dimensional response surface plots and two-dimensional contour plots of OD were constructed based on Statistica 7.0 software. Three-dimensional plots can represent only the relationship between response and two factors. Significance test of binomial regression model coefficients indicated that X_1, X_3 had very significant influence on indexes ($P < 0.01$ in each case), analysis of variance as Table 3. Therefore, the first fixed an independent variable, X_1 and X_2 was the coded levels of the independent variable respectively and subsequently was put into polynomial equations. Figure 1 portray three-dimensional response surface and two-dimensional contour plots for OD to X_1 and X_3; Figure 2 portray three-dimensional response surface and two-dimensional contour plots for OD to X_1 and X_2; Figure 3 portray three-dimensional response surface and two-dimensional contour plots for OD to X_2 and X_3.

According to the above visual analysis of three-dimensional response surface plots and contour plots for OD, The optimized index of the preparation included: The HPMC content of swelling layer (X_2) was 4~5% (g/ml), the coating weight gain (X_1) was 21~23%, and the weight gain percent of swelling layer was 5-7%.

Optimization of process validation

The optimum prescription of GSP was determined according

Table 3: Significance test of binomial regression model coefficient table for OD

Source of variance	Squares	Freedom	Mean square	*F*	*P*
X_1	0.179292	1	0.179292	14.74101	0.006379*
X^2	0.784953	1	0.784953	64.53715	0.000089*
X_2	0.003582	1	0.003582	0.29448	0.604202
X_2^2	0.117664	1	0.117664	9.67405	0.017074
X_3	0.004642	1	0.004642	0.38165	0.556279
X_{32}	0.182705	1	0.182705	15.02157	0.006086*
X_1X_2	0.073191	1	0.073191	6.01759	0.043909
X_1X_3	0.017819	1	0.017819	1.46502	0.265413
X_2X_3	0.070827	1	0.070827	5.82326	0.046559

OD: Overall desirability, $P<0.01$*

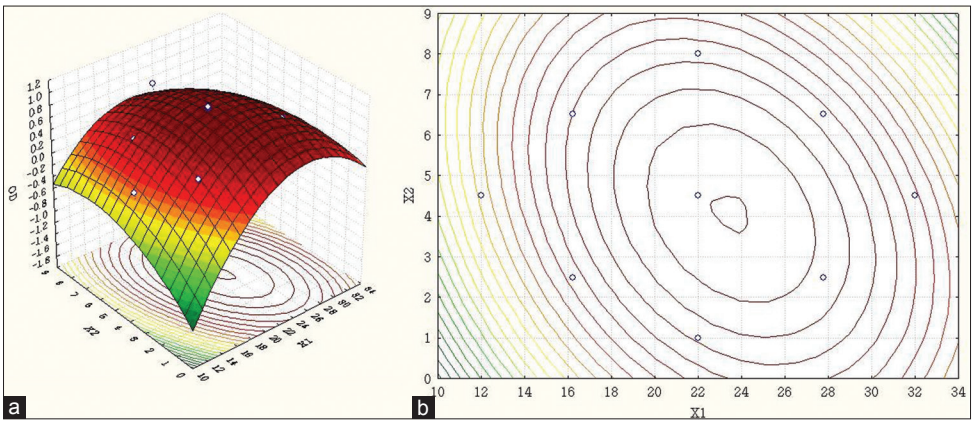

Figure 1: (a) Response surface plot showing the influence of X_1 and X_3; (b) the corresponding contour plot

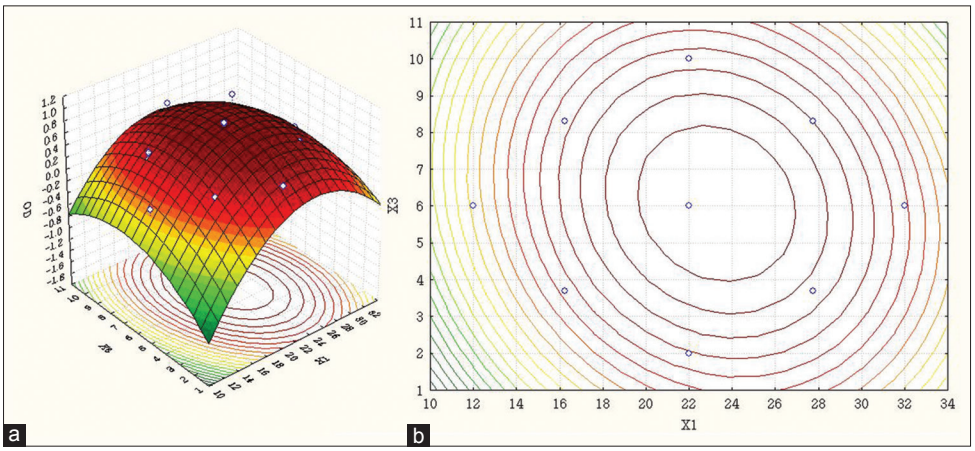

Figure 2: (a) Response surface plot showing the influence of X_1 and X_2; (b) the corresponding contour plot

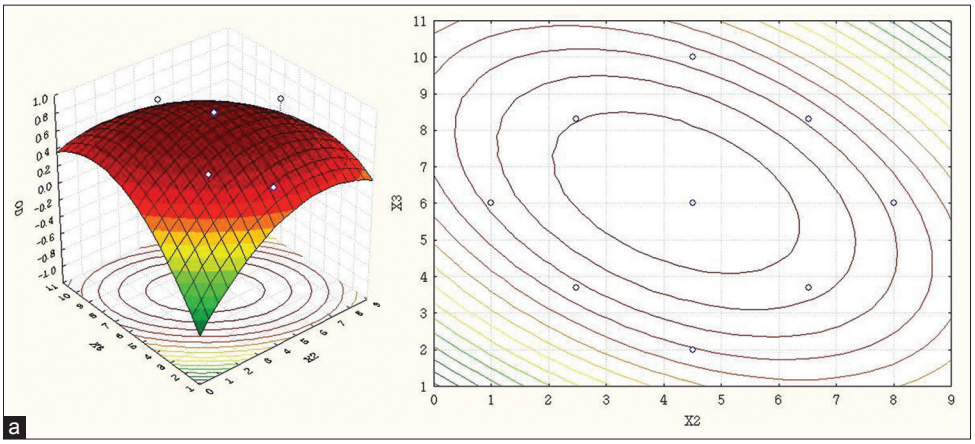

Figure 3: (a) Response surface plot showing the influence of X_2 and X_3; (b) the corresponding contour plot

to central composite design combining with the result of investigating pre-experiment single factor and practical operability, the optimized formula: Coating weight gain is 22%, the concentration of HPMC in swelling layer is 4.5% (g/ml), the percentage of swelling layer weight gain is 6%.

To determine the degree of coincidence between the models set up in star point design experiments and practical results, using farther checking experiments to test the accuracy. Prepare GSP with optimized prescription and technological parameter, then determine the release and roundness of each composition and comparing them with predicted value. As we can see in Table 4, the relative deviation of theory forecast and measured were <5% states that the best prescription condition of the experiment is reasonable, better stability and repeatability

states that central composite design (CCD)-response surface methods can be applied in optimizing coating prescription of GSP.

Validation of the assay

Selection of index components

Biological activity of naringin mainly related to its effect on metabolic enzymes in plasma and liver, it can enhance the enzyme activity such as glutathione reductase, superoxide dismutase and catalase *in vivo*.[23-25] Meanwhile, the drug-metabolizing enzyme CYP450 and P-glycoprotein *in vivo* can be competitively inhibited.[26,27] Hence, the biological bioavailability of drug *in vivo* which could be metabolized and efflux was improved.

Ganershu sustained-release pellet were composed of four effective parts, saponins and flavonoids as main active parts. The results of studying on the intermediate showed that naringin and its metabolites simultaneously existed in rats, mainly in the form of naringenin. The therapeutic effect of GSP was based on naringenin. Therefore, naringenin was selected as determination indicators of GSP *in vivo*.[28-31]

Investigation of specificity

The selectivity of the method was evaluated by analyzing blank samples prior to plasma samples. We could see that the chromatograms were free of interfering peaks at the retention times of luteolin (IS) and naringenin.

Linearity

The linearity of naringenin was assessed by linear regression of calibration curves based on peak area ratios of naringenin to the internal standard. The standard curves of naringenin in plasma samples were linear in the concentration ranges (0.035-6.181 μg/ml) examined.

Table 4: Measured, forecast and deviation of each evaluation index at optimized conditions (*n*=3)

Evaluation index	Forecasted value	Measured value	Deviation (%)
Cumulative release rate (1 h)/%	18.65	18.84	−1.02
Cumulative release rate (4 h)/%	50.11	49.70	0.82
Cumulative release rate (8 h)/%	75.06	74.24	1.09
Roundness/°	18	18	0

The regression equation for naringenin was

$$Y = 7.655X - 0.0239 \ (r = 0.996)$$

Where y was peak area ratios of naringenin to IS in plasma samples and x was concentration of naringenin.

Precision and accuracy

The blank plasmas of beagles were added to amounts of naringenin standard solutions. Samples were treated, according to sample preparation. Precision studies were performed by analysis of three different concentrations of naringenin 5 times on the same day (intraday) and 5 consecutive days (interday). The precision and accuracy were also shown in Table 5. The precisions of naringenin calculated as the RSD at three concentrations were lower than 5%, and the accuracy was also within 5% for samples for quality control. The results demonstrated that the precision and accuracy of this method was acceptable. The method was proved to be suitable for the naringenin in plasmas, which offers advantages of high sensitivity and selectivity.

Recovery

The recoveries for naringenin were also listed in Table 5 and showed an overall mean percent recovery of 82.82%, 85.94% and 98.32%, respectively.

Pharmacokinetics of naringenin in Ganershu normal pellet and Ganershu sustained-release pellet after oral dosing

Response surface methodology is a rapid technique used to empirically derive a functional relationship between an experimental response and a set of input variables in the development and optimization of drug delivery systems. In this work, central composite design was used simultaneously to study the effect of the three process parameters involved in the preparation of GSPs against three response variables. CCD is a response surface design, which provides information on direct effects, pair-wise interaction effects, and curvilinear variable effects and is widely used for formulation and process optimization in the field of pharmaceutics.[32,33] In this paper, it was used to optimized of the coating prescription of GSP.

Table 5: Precision and accuracy and recovery of naringenin in plasma samples (*n*=6)

Analytes	Concentration (μg/ml)	Precision (RSD %)		Accuracy (RE %)	Recovery (%)
		Interday	Intraday		
Naringenin	0.115	2.47	1.81	−4.55	92.82
	0.909	4.58	3.37	+1.43	85.94
	8.181	1.24	0.31	−2.61	98.32

RSD: Relative standard deviation; RE: Relative error

Coating is a vital stage in the formulation of pharmaceutical dosage form where ultimate objective is to achieve superior aesthetic quality (like color, texture and taste) and to modify drug release characteristics. Frequently, the coated was to modify drug release kinetics. Thus, the product performance directly correlates with critical film coating quality attributes.[34]

We assessed the oral bioavailability of the naringenin in Ganershu normal pellet (GNP) and GSP by Beagles as shown in Figure 4. A summary of the statistical analysis is shown in Table 6. The average values for maximum concentration and time to maximum concentration after oral administration of GNP were 10.067 μg/mL and 4.021 h, respectively, while those after oral administration of the GSP were 8.312 μg/mL and 7.005 h, respectively. In Pharmacokinetics of GNP and GSP after oral dosing in beagle dogs, the recommended mean residence time $(MRT)_{0-\infty}$ of naringenin in blood of GNP is 3.635 h and it means is effective in maintaining short-term in solid organs (such as liver). So sustained release preparations are urgently needed. It has been proposed that therapy requires the use of a sustained release formulation for prolonged action and to improve patient compliance. Multiparticulate dosage forms are desirable drug delivery systems owing to a number of advantages over single unit dosage forms, such as better control of the gastric transit time and associated drug absorption.[35]

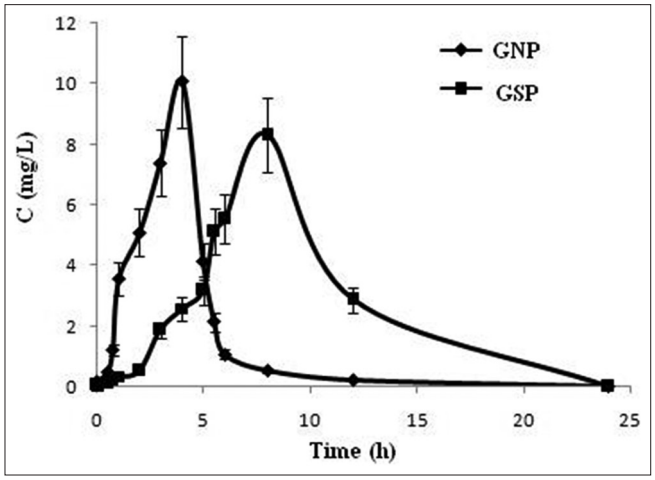

Figure 4: The concentration-time curve of naringenin in Ganershu normal pellet and Ganershu sustained-release pellet

Table 6: Pharmacokinetic parameters of GSP and GNP (*n*=6)

Pharmacokinetic parameters	Units	GSP	GNP
AUC_{0-t}	U/L·h	52.67±4.791	38.8114.536
$AUC_{0-\infty}$	L^{-1}·h	52.64C4.783	38.0034.533
MRT_{0-t}	h	8.62840.207	3.63540.015
$MRT_{0-\infty}$	h	4.598±0.121	2.218±1.694
T_{max}	h	7.005±0.035	4.021±0.329
C_{max}	mg/L	8.312±0.313	10.067±0.518

GSP: Ganershu sustained-release pellets, GNP: Ganershu normal pellets, AUC: Area under the curve, MRT: Mean residence time

Sustained release dosage forms are designed to achieve a prolonged therapeutic effect by continuously releasing the medication over an extended period of time after administration of a single dose. This period is measured in hours and critically depends on the residence time of the dosage form in the gastrointestinal tract. To extend the $MRT_{0-\infty}$ of naringenin, the longer-releasing formulation was needed to use. Therefore, the sustained-release pellets has been successfully used to deliver it in beagle dogs models and showed up to 8.628 h of stable drug release after a single pellet oral. Besides that, the area under the curve $(AUC)_{0-\infty}$ of naringenin in GSP was 1.38 times greater than that of GNP. The relative bioavailability of GSP increased with the sustained-release coating of the GNP. Meanwhile, *T*max of GSP was prolonged for about 74%. Therefore, we enhanced the oral bioavailability of Ganershu by preparing the GSP, which had the sustained dissolution and improved the potential of it for clinical application.

Traditional Chinese medicine compound recipe is a precious heritage of Chinese medicine and has made a prominent contribution to the prosperity of the Chinese nation. Now they are increasingly being understood and accepted by more and more people in the world. In recent years, it is becoming so much in vogue because of the consumers' anxiety for the undesired secondary effects of synthetic drugs and the "green movement," which has been resurgent in Europe, North America and Australia. In this study, the bottom-spraying process of fluidized bed technology was applied to prepare a sustained-release pellet of Ganershu. The multiparticulate dosage form consists of the small discrete units. Together, these units provide the overall desired controlled release of the formulation. It composed of principal components, deputy components, adjuvant components and the guide components. The principal component (naringin) is a substance that provides the main therapeutic effects, while the deputy, adjuvant and guide components assist the therapeutic actions of the principal components. We combined them properly to make it sustained dissolution and improved the bioavailability.

CONCLUSIONS

Central composite design was used simultaneously to study the effect of the three process parameters involved in the preparation of GSPs. The naringenin as an index, the $AUC_{0-\infty}$ of naringenin in GSP was 1.38 times greater

than that of GNP in in the beagle dog models. Meanwhile, *T*max of GSP was prolonged for about 74%. Based on those results, it can indicate that we enhanced the oral bioavailability of Ganershu by preparing the GSP, which had the sustained dissolution and improved the potential of it for clinical application.

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