Hindawi

*Research Article*

# Study on Central Composite Design Method to Optimize the Preparation Process of Chrysophanol-Pluronic F127 Nanomicelles and Pharmacokinetics

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*Objective*. In order to find the best process for the preparation of Chrysophanol-Pluronic F127 nanomicelles in the laboratory, the preparation of Chrysophanol-Pluronic F127 nanomicelles was optimized using the central composite design method. In order to investigate the properties of the prepared Chrysophanol-Pluronic F127 nanomicelles, physicochemical properties were examined and in vitro dissolution experiments were performed, and the pharmacokinetics of Chrysophanol and Chrysophanol-Pluronic F127 nanomicelles were investigated in rabbits. *Methods*. In the preexperimental study, the best physical solubilization method and organic solvent for Chrysophanol were selected. The ratio of drug dosage to excipient dosage and the amount of organic solvents were evaluated by single-factor test. Based on the single-factor test, the optimal prescription was obtained by screening the formulation and optimizing the preparation process using the central composite design method with the encapsulation efficiency as the index. Chrysophanol-Pluronic F127 nanomicelles were prepared according to the optimal prescription, and their particle size, potential, appearance, and in vitro release experiments were carried out. Chrysophanol and Chrysophanol-Pluronic F127 nanomicelles were injected intravenously through the ear margins to rabbits, and the drug concentrations in the blood were measured at different time points by HPLC. The obtained blood concentration data were fitted with PK Solver 2.0 program to obtain pharmacokinetic parameters. *Results*. In the preexperimental study, ultrasonic method was selected as the physical solubilization method, and acetone was selected as the organic solvent. In single-factor test, the highest encapsulation efficiency was achieved when the ratio of drug dosage to excipient dosage was $1:15$; the highest encapsulation efficiency was achieved when the amount of organic solvent (acetone) was 8 mL. The equation fitted to the model for the optimized prescription by the central composite design method is as follows: $R1 = -166.93629 + 16.86478A + 32.55582B - 0.169750AB - 0.482675^2 - 2.25797B^2 (R^2 = 0.9457)$. The best prescription for the preparation of Chrysophanol-Pluronic F127 nanomicelles was obtained in the ratio of drug dosage to excipient dosage of $1:16.309$ and the dosage of organic solvent was 6.595 mL. The prepared nanomicelles have a particle size of 152.8 nm and a potential of -23.9 mV. It was observed by transmission electron microscope that the prepared nanomicelles are uniform and spherical in appearance. The drug metabolism of Chrysophanol and nanomicelles in rabbits conforms to the two-compartment open model, and both of them show linear kinetics in the drug dose range. $T(1/2^{\alpha})$ was $0.31 \pm 0.21$ h and $0.47 \pm 0.35$ h, and $T(1/2^{\beta})$ was $2.06 \pm 1.14$ h and $7.72 \pm 2.04$ h for Chrysophanol and Chrysophanol-Pluronic F127 nanomicelles, respectively. *Conclusions*. The adopted central composite design method can well optimize the prescription process of Chrysophanol-Pluronic F127 nanomicelles prepared by dialysis, and the method is simple and easy to be prepared in the laboratory. The prepared nanomicelles have uniform particle size and good zeta potential and appear as uniform black spherical shape under transmission electron microscopy. In vitro release studies showed that the Chrysophanol-Pluronic F127 nanomicelles released significantly better than Chrysophanol. The results of pharmacokinetics of Chrysophanol and Chrysophanol-Pluronic F127 nanomicelles in rabbits showed that the Chrysophanol-Pluronic F127 nanomicelles did not change the metabolic process of the drug in vivo but could stay in vivo for a longer period of time and exert longer effects. It is hoped that this study can provide a laboratory basis for the preparation of Chrysophanol-Pluronic F127 nanomicelles and a reference for further in vivo studies of Chrysophanol.

# 1. Introduction

Chrysophanol, chemically known as 1,8-dihydroxy-3-methyl-anthraquinone, is an anthraquinone compound extracted from traditional Chinese medicines such as Chinese rhubarb and Giant Knotweed Rhizome. Chrysophanol has a molecular weight of 254.23 and contains multiple carbonyl and hydroxyl groups as well as an anthraquinone tricyclic aromatic structure [1]. Recent studies have shown that Chrysophanol have various pharmacological effects, such as anticancer and neuronal protection [2, 3], but its poor water solubility and low bioavailability make it difficult to use in clinical practice. Therefore, various studies on Chrysophanol dosage forms have emerged [4, 5], but fewer studies have been conducted on Chrysophanol micelles dosage forms [6], and the issue of multifactorial interactions in recent studies on Chrysophanol dosage forms remains to be addressed [7]. Nanomicelles are of high research value and have been studied by many scholars because of their absorption-promoting and bioavailability-enhancing effects [8–10]. In this study, Pluronic F127 [11] was used as a carrier to prepare Chrysophanol-Pluronic F127 nanomicelles and perform in vivo pharmacokinetic studies in rabbits, aiming to establish a method to optimize the preparation of nanomicelles by dialysis using the central composite design method, in order to provide a reference for the clinical application of Chrysophanol.

# 2. Materials and Methods

2.1. Materials. HPLC (Waters e2695, Waters 2998) (Waters, US)

UV-visible spectrophotometer (LebTech, US)

Hj-4D digital display constant temperature velocimetry magnetic heating stirrer (Jiangsu Zhengji Instrument Co., Ltd., China)

SiGMA 3K30 high speed refrigerated centrifuge (Sigma-Aldrich, US)

H7650 transmission electron microscope (Hitachi, Japan)

ZETASIZER nanoseries (Malvern Instruments Co., Ltd., UK)

Vortex oscillator FSH-2 (Jintan Shengwei Experimental Instrument Factory, China)

Ultrasonic generator KQ5200DV (Kunshan Ultrasonic Instruments Co., China)

Dialysis bags MD34MM (diameter: 22 m, retained molecular weight: 1000) (Viskase, US)

Chrysophanol (Chengdu Lemeitian Pharmaceutical Technology Co., Ltd., China)

Pluronic F127 (Sigma-Aldrich, US)

New Zealand rabbit (Beijing Jinmuyang Experimental Animal Breeding Co., China)

# 3. Methods

## 3.1. Preparation of Micelles

3.1.1. (1) Pretreatment of Dialysis Bags [12]. The dialysis bags were cut into small sections of appropriate length (10-20 cm), and the cut bags were boiled in a large volume of 2% (w/v) sodium bicarbonate and 1 mmol/L EDTA (pH 8.0) for 10 min. The boiled dialysis bags were thoroughly washed with distilled water. Boil the cleaned dialysis bags in 1 mmol/L EDTA (pH 8.0) for 10 min. After cooling, store in a 4°C refrigerator and ensure that the dialysis bags are always submerged in the solution. Before use, fill the dialysis bag with water and drain it, wash it well, and take it with gloves.

### 3.1.2. Preexperimental Study

(1) Selection of Physical Solubilization Methods. The four physical solubilization methods of stirring, magnetic stirring, water bath shaking, and ultrasonic were set up, and the method was selected by observing the dissolution time of Chrysophanol in the solution and the measured absorbance.

(1) Precisely weigh 2.0 mg of Chrysophanol and add 10 mL of anhydrous ethanol; stirring, magnetic stirring, water bath shaking, and ultrasonic treatment were performed to set up three parallel experiments to observe the dissolution time of Chrysophanol in solution

(2) Precisely weigh 1.0 mg of Chrysophanol in a 50 mL volumetric flask and fix the volume with anhydrous ethanol to obtain 20 $\mu g\cdot mL^{-1}$ of Chrysophanol stock solution. Transfer 12.5 mL of the reserve solution into a 25 mL volumetric flask and dilute to the scale with anhydrous ethanol to obtain a standard solution at a concentration of 10 $\mu g\cdot mL^{-1}$. The absorbance was measured in three parallel experiments with stirring, magnetic stirring, water bath shaking, and physical solubilization by ultrasonic method for 20 min, respectively

(2) Selection of Organic Solvents. Anhydrous ethanol, chloroform, dichloromethane, and acetone were set as four organic solvents to prepare Chrysophanol-Pluronic F127 nanomicelles by the dialysis method described in Section 3.1.3, and the encapsulation efficiency of nanomicelles was used as the index.

### 3.1.3. Preparation Process of Chrysophanol-Pluronic F127 Nanomicelles. The nanomicelles were prepared by dialysis method [13–15]. The appropriate amount of Chrysophanol and the appropriate amount of Pluronic F127 were precisely weighed and dissolved with the appropriate amount of acetone, and after the solution was clarified, it was slowly injected into 10 mL of ultrapure water with a syringe and magnetically stirred at 1000 $r\cdot min^{-1}$ for 60 min, and the obtained solution was injected into the treated dialysis bags for 24 h. After 24 h, the solution in the dialysis bag was removed to a centrifuge tube, the free Chrysophanol was dispersed by centrifugation in a high-speed centrifuge, and the supernatant was the prepared nanomicelles. Three sets of parallel experiments were established, and encapsulation efficiency of the prepared nanomicelles was measured.

### 3.1.4. Determination Method and Standard Curve Construction

(1) The content of Chrysophanol was determined by UV spectrophotometric method [16–18]. Referring to the Chinese Pharmacopoeia 2020 edition, the detection wavelength under the determination of Chrysophanol content in one section is 254 nm, so 254 nm was determined as the detection wavelength of Chrysophanol in this study. Pluronic F127 was weighed precisely, dissolved in anhydrous ethanol and diluted to a certain multiple, and scanned in the wavelength range of 200-600 nm with anhydrous ethanol as the blank control

(2) Precisely weigh 1.00 mg of Chrysophanol in a 50.00 mL volumetric flask, and fix the volume with anhydrous ethanol to obtain a reserve solution of Chrysophanol at a concentration of 20.00 $\mu$g·mL$^{-1}$. The reserve solution was transferred into a 25.00 mL volumetric flask and diluted to the scale with anhydrous ethanol to obtain a series of standard solutions with concentrations of 0.10, 0.25, 0.50, 1.00, 2.50, 5.00, and 10.00 $\mu$g·mL$^{-1}$. After measuring the absorbance of the samples at 254 nm, a linear regression was performed by least squares to establish the regression equation

### 3.1.5. Single-Factor Test

(1) The nanomicelles were prepared according to the dialysis method described in Section 3.1.3. The ratio of drug dose to excipient dose was set at 1 : 5, 1 : 10, 1 : 15, 1 : 20, and 1 : 25, i.e., 25.00 mg, 50.00 mg, 75.00 mg, 100.00 mg, and 125.00 mg of Pluronic F127 for 5.00 mg of Chrysophanol, respectively. Three sets of parallel experiments were set up, and the encapsulation efficiency was measured

(2) The nanomicelles were prepared according to the dialysis method described in Section 3.1.3. The organic solvent (acetone) volume conditions were set to 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL. Three groups of parallel experiments were set and the encapsulation efficiency was measured

### 3.1.6. Optimization of Prescription by Central Composite Design Method. 
The amount of organic solvent(acetone) and the ratio of drug amount to excipient amount were selected as two conditions for prescription optimization using the central composite design response surface method (CCD design method) [19–21] using Design Expert 12 software [22].

### 3.2. Study of Particle Size, Potential and Appearance of Chrysophanol-Pluronic F127 Nanomicelles. 
The particle size and potential of the prepared nanomicelles were determined using a Malvern particle size meter. The appearance of the prepared micelles was observed by transmission electron microscopy.
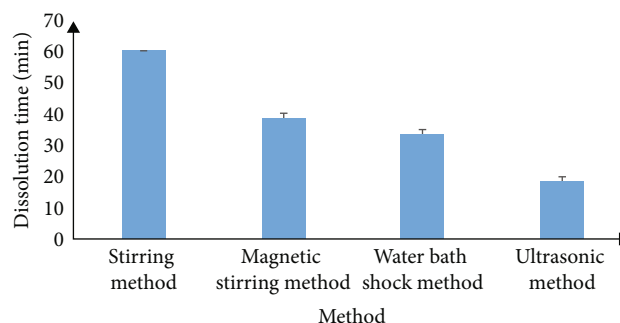


FIGURE 1: Effect of different physical solubilization methods on the dissolution time of Chrysophanol. The error bars represent the standard deviation of three independent sample measurements ($n = 3$).
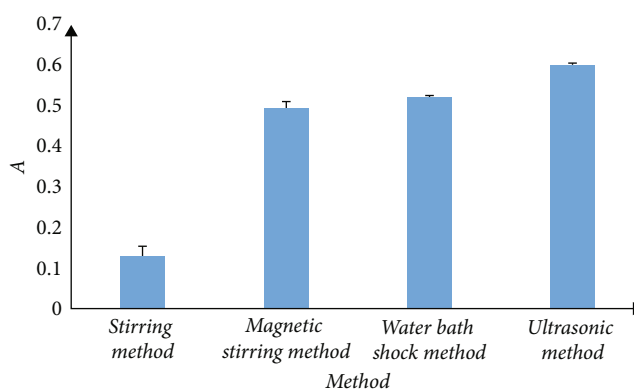


FIGURE 2: Effect of different physical solubilization methods on the absorbance of Chrysophanol solution. The error bars represent the standard deviation of three independent sample measurements ($n = 3$).
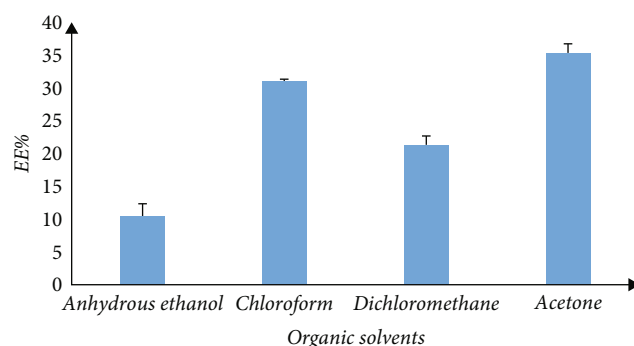


FIGURE 3: Effect of different physical solubilization methods on the encapsulation efficiency of Chrysophanol nanomicelles. The error bars represent the standard deviation of three independent sample measurements ($n = 3$).

### 3.3. In Vitro Release Studies. 
The in vitro release behavior of nanomicelles was investigated by the dialysis bag method using 900 mL of 0.5% sodium dodecyl sulfate as the release medium, and the dialysis bag treatment method was the same as that under Section 3.1.1. Chrysophanol and Chrysophanol-Pluronic F127 nanomicelles were placed in
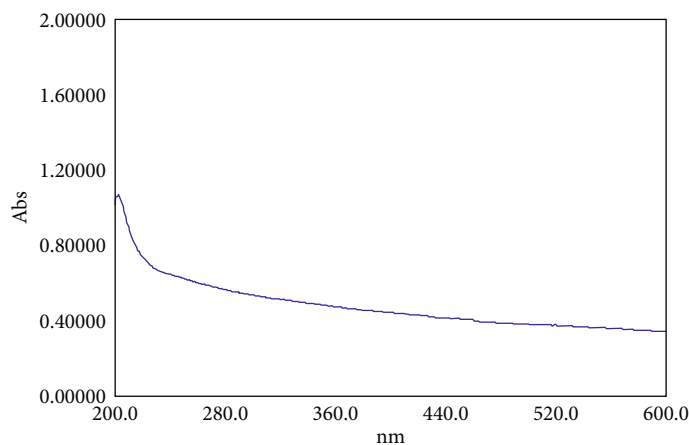
FIGURE 4: The UV spectrophotometer wavelength scan spectrum of Pluronic F127.

dialysis bags, tied to a paddle, and then placed in a dissolution cup with the release medium, and the solution temperature was controlled at 3°C by an intelligent drug dissolution instrument, and the speed was set at $100 \, \text{r·min}^{-1}$. The samples were diluted to a certain multiple with anhydrous ethanol, and the absorbance of the nanomicelles was determined by UV method, and the absorbance was substituted into the standard curve regression equation to calculate the Chry concentration and plot the dissolution curve.

① Linearity Range Investigation

The standard solutions were prepared with 0.5% sodium dodecyl sulfate at concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5, and $10 \, \mu\text{g·mL}^{-1}$. The absorbance was determined by UV method, and linear regression was performed by least squares method to obtain the standard curves.

*3.4. In Vivo Pharmacokinetic Study in Rabbits.* The experimental animal experiments and posttest treatments in this study are conducted with the approval of the Experimental Animal Ethics Review Board of Hebei North University, Zhangjiakou, China.

*3.4.1. Chromatographic Conditions.* The chromatographic column is Hypersil ODS2 (4.6 mm × 250 mm, 5 μm), mobile phase: methanol - 0.1% phosphoric acid (85 : 15), flow rate: $1 \, \text{mL·min}^{-1}$, detection wavelength: 254 nm, column temperature: 30°C, and injection volume: 20 μL.

*3.4.2. Treatment of Chrysophanol Control Group.* The Chrysophanol was precisely weighed, dissolved in N,N-dimethylformamide, then added with polysorbate 80, and diluted with saline (N,N-dimethylformamide-polysorbate 80-saline (1 : 1 : 8)) [23].

*3.4.3. Experimental Procedure and Blood Sample Processing.* Twelve healthy rabbits of both sexes weighing 1.5-2.5 kg were randomly divided into two groups and fasted for 12 h before the experiment. One group was injected with Chrysophanol control group solution (the control solution of Chrysophanol was prepared in Section 3.4.2.) $7.0 \, \text{mg·kg}^{-1}$ intravenously at the ear margin vein, and the other group was injected with Chrysophanol-Pluronic F127 nanomicelles
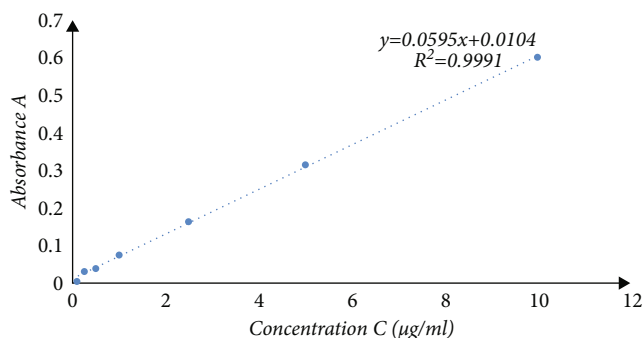


FIGURE 5: The standard curve of Chrysophanol.

(equivalent to Chrysophanol $7.0 \, \text{mg·kg}^{-1}$) intravenously at the ear margin vein. At 0.083, 0.25, 0.5, 1, 2, 4, 8, and 12 h after administration, 1 mL of blood was drawn from the heart of the rabbits. The blood was placed in EDTA-coated tubes and centrifuged at $10{,}000 \, \text{r·min}^{-1}$ for 10 min. 100 μL of supernatant plasma was collected and stored in a refrigerator at -80°C until use. Plasma samples were processed using the ethyl acetate resolution method [24]. After centrifugation, 100 μL of the supernatant was placed in a 1 mL centrifuge tube, and 1 mL of ethyl acetate was added, vortexed for 5 min, and centrifuged at $12000 \, \text{r·min}^{-1}$ for 10 min (4°C). 900 μL of the upper layer of ethyl acetate was aspirated and placed in a new centrifuge tube and blown dry with nitrogen, 200 μL of mobile phase was added and redissolved and vortexed for 5 min, and the supernatant was aspirated into the sample. The supernatant was centrifuged at $12000 \, \text{r·min}^{-1}$ for 10 min (4°C), aspirated, and reserved.

*3.4.4. Establishment of Standard Curve.* Add 100 μL of rabbit plasma in a 1 mL centrifuge tube with 10 μL of Chrysophanol standard solution (1.00, 1.50, 2.00, 5.00, 10.00, 15.00, 20.00, 50.00, 100.00, and $150.00 \, \mu\text{g·mL}^{-1}$); then add 1 mL of ethyl acetate extract, vortex and mix for 5 min, and centrifuge at $12000 \, \text{r·min}^{-1}$ for 10 min (4°C). Aspirate 900 μL of the upper layer of ethyl acetate into a new centrifuge tube, blown dry under nitrogen, add 200 μL of mobile phase to redissolve, vortex for 5 min, centrifuge at $12000 \, \text{r·min}^{-1}$ for

TABLE 1: The table of optimization prescription by CCD design method.

| Std | Run | Factor 1 A:A<br>The ratio of drug dose to excipient dose | Factor 2 B:B<br>Amount of organic solvent | Response 1 R1<br>EE% |
| --- | --- | --- | --- | --- |
| 9 | 1 | 15 | 8 | 73 |
| 4 | 2 | 20 | 10 | 47.67 |
| 12 | 3 | 15 | 8 | 73 |
| 8 | 4 | 15 | 10.8284 | 34.15 |
| 10 | 5 | 15 | 8 | 73 |
| 11 | 6 | 15 | 8 | 73 |
| 3 | 7 | 10 | 10 | 35.76 |
| 5 | 8 | 7.92893 | 8 | 46.58 |
| 13 | 9 | 15 | 8 | 73 |
| 1 | 10 | 10 | 6 | 49.92 |
| 6 | 11 | 22.0711 | 8 | 53.97 |
| 2 | 12 | 20 | 6 | 68.62 |
| 7 | 13 | 15 | 5.17157 | 78.54 |

TABLE 2: Model significance results of optimization prescription by CCD design method.

| Source | Sum of squares | df | Mean square | $F$-value | $P$ value | |
| --- | --- | --- | --- | --- | --- | --- |
| Model | 2826.57 | 5 | 565.31 | 24.37 | 0.0003 | Significant |
| A-A | 210.75 | 1 | 210.75 | 9.09 | 0.0195 | |
| B-B | 1197.73 | 1 | 1197.73 | 51.64 | 0.0002 | |
| AB | 11.53 | 1 | 11.53 | 0.4969 | 0.5036 | |
| $A^2$ | 1012.94 | 1 | 1012.94 | 43.67 | 0.0003 | |
| $B^2$ | 567.48 | 1 | 567.48 | 24.47 | 0.0017 | |
| Residual | 162.36 | 7 | 23.19 | | | |
| Lack of fit | 162.36 | 3 | 54.12 | | | |
| Pure error | 0.0000 | 4 | 0.0000 | | | |
| Cor total | 2988.93 | 12 | | | | |

10 min (4°C), and aspirate the supernatant. According to the chromatographic conditions in Section 3.4.1, the supernatant was determined by HPLC and a standard curve was established.

*3.4.5. Data Processing.* Pharmacokinetic parameters data were fitted according to the PK Solver 2.0 program [25], and the fitted model was evaluated and pharmacokinetic parameters were calculated.

## 4. Results and Discussion

*4.1. Preexperimental Study and Standard Curve Construction*

(1) In the preexperimental study, the dissolution time of Chrysophanol in solution was $60 \pm 1$, $38.33 \pm 5$, $33.33 \pm 5$, and $18.33 \pm 5$ min after stirring, magnetic stirring, water bath shaking, and ultrasonication. The absorbance measured by the four methods was $0.13 \pm 0.05$, $0.49 \pm 0.03$, $0.52 \pm 0.01$, and $0.60 \pm 0.01$. The results are shown in Figures 1 and 2. Ultrasonication was chosen as the physical solubilization method for this study because it had the shortest dissolution time and the largest absorbance

(2) Chrysophanol-Pluronic F127 nanomicelles were prepared by dialysis with four organic solvents, namely, anhydrous ethanol, chloroform, dichloromethane, and acetone. The encapsulation efficiency of the prepared nanomicelles was $10.426 \pm 3.673\%$, $30.947 \pm 0.62\%$, $21.287 \pm 2.58\%$, and $35.307 \pm 2.34\%$, and the results are shown in Figure 3. The acetone was chosen as the organic solvent for this study because the encapsulation efficiency of the prepared nanomicelles was the largest

(3) After dissolving Pluronic F127 in anhydrous ethanol and diluting it by a certain multiple, the UV spectrophotometer was scanned in the wavelength range of 200~600 nm using anhydrous ethanol as a blank control. According to the full wavelength scan spectrum of UV spectrophotometer in Figure 4, the maximum absorption wavelength of Pluronic F127 was at 200 nm, which did not interfere with Chrysophanol

Factor coding:Actual

R1 (□□□)
Design points
● Above surface
○ Below surface
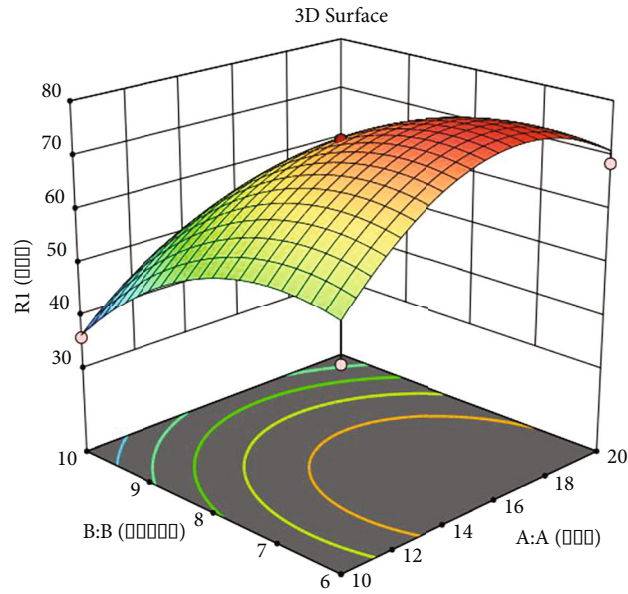34.15 ▮▮▮▮ 78.54

X1=A:A
X2=B:B



FIGURE 6: 3D effect surface fitting model for optimal prescription by CCD design method.

Factor coding:Actual

R1 (□□□)
● Design points

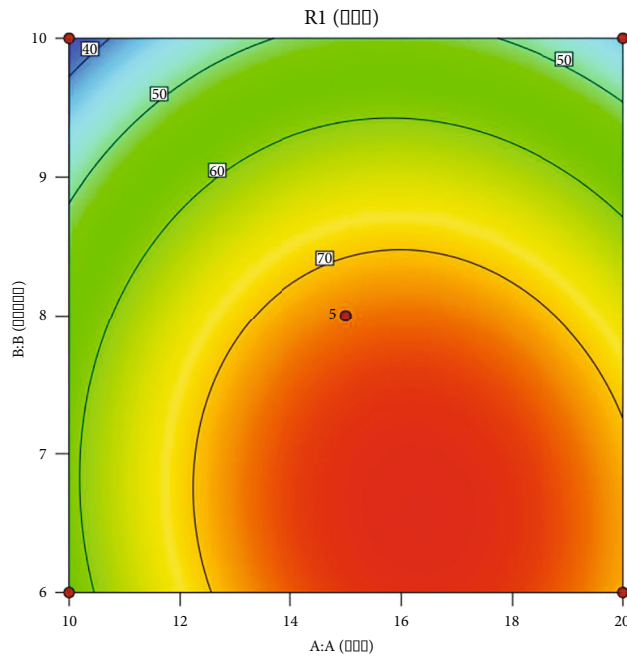34.15 ▮▮▮▮ 78.54
X1=A:A
X2=B:B



FIGURE 7: 2D effect surface fitting model for optimal prescription by CCD design method.

TABLE 3: Optimum preparation conditions.

| Number | A | B | R1 | Desirability | |
| --- | --- | --- | --- | --- | --- |
| 1 | 16.312 | 6.596 | 77.967 | 0.987 | Selected |

Results

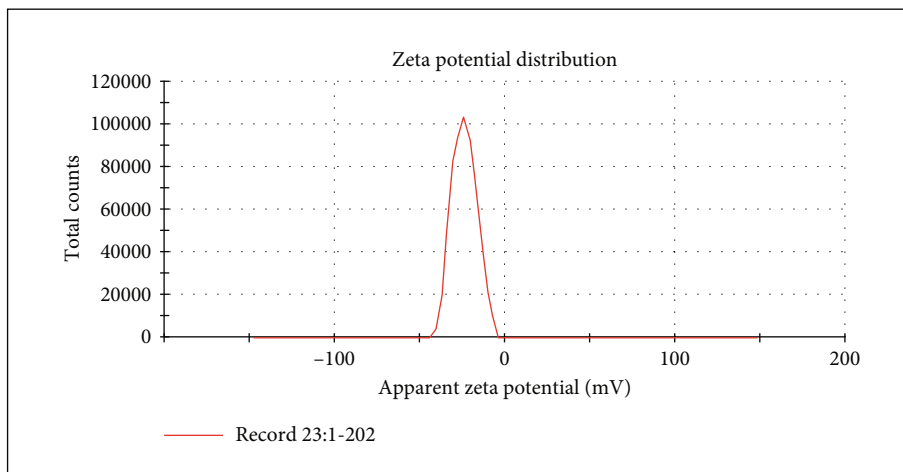| | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|
| Zeta potential (mV): −23.9 | | | |
| | Peak 1: −23.9 | 100.0 | 7.17 |
| Zeta deviation (mV): 7.17 | | | |
| | Peak 2: 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 0.0218 | | | |
| | Peak 3: 0.00 | 0.0 | 0.00 |
| Result quality Good | | | |



FIGURE 8: The zeta potential distribution of Chrysophanol-Pluronic F127 nanomicelles. The maximum peak is at -23.9 mV.

Results

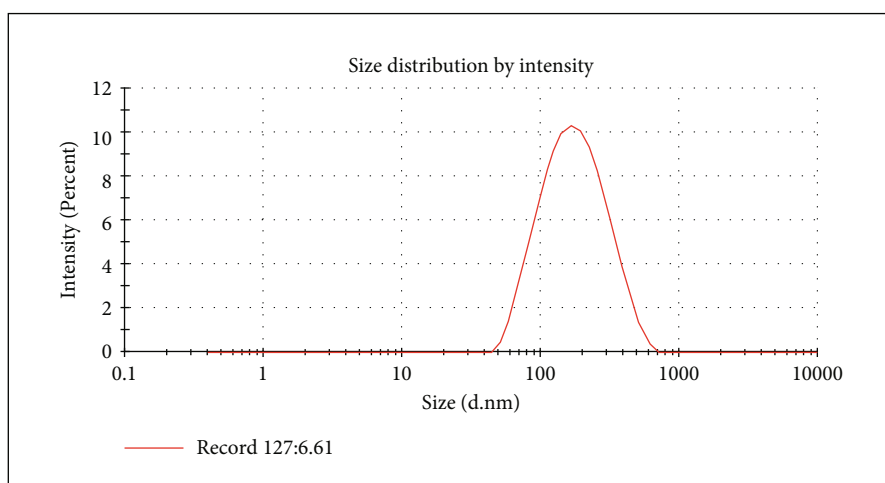| | Size (d.n... | % Intensity: | St Dev (d.n... |
|---|---|---|---|
| Z-Average (d.nm): 152.8 | | | |
| | Peak 1: 194.4 | 100.0 | 103.9 |
| Pdl: 0.225 | | | |
| | Peak 2: 0.000 | 0.0 | 0.000 |
| Intercept: 0.887 | | | |
| | Peak 3: 0.000 | 0.0 | 0.000 |
| Result quality Good | | | |



FIGURE 9: The particle size distribution of Chrysophanol-Pluronic F127 nanomicelles. The PDI is 0.225 and the maximum peak is at 194.4 nm.
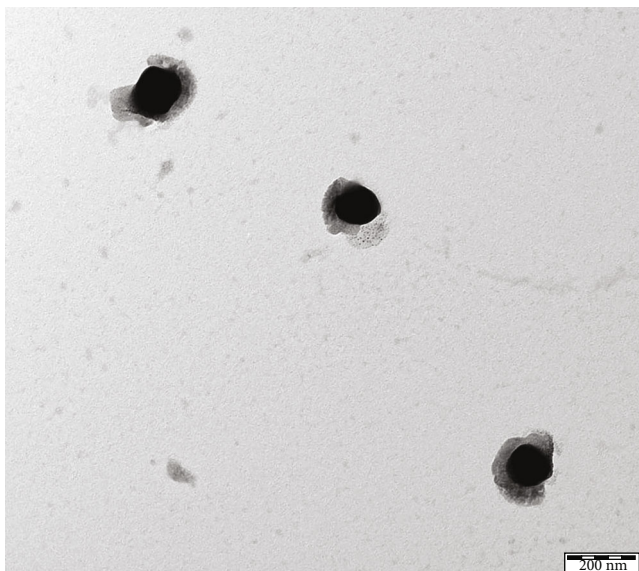
FIGURE 10: The morphology of Chrysophanol-Pluronic F127 nanomicelles under transmission electron microscopy using 200 nm as a scale.
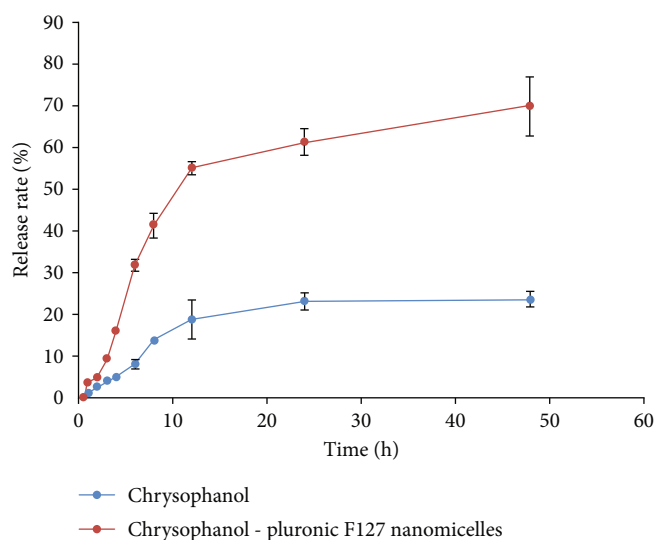


FIGURE 11: The in vitro dissolution curve of Chrysophanol-Pluronic F127 nanomicelles and Chrysophanol. The error bars represent the standard deviation of three independent sample measurements ($n = 3$).

The equation of the standard curve for linear regression using least squares is Equation (1), and the results are shown in Figure 5.

$$y = 0.0595x + 0.0104 \left(R^2 = 0.9991\right). \quad (1)$$

### 4.2. Single-Factor Test and Prescription Optimization

#### 4.2.1. Single-Factor Test

(1) Chrysophanol-Pluronic F127 nanomicelles were pre-pared by dialysis when the ratio of drug amount to excipient amount was set at 1:5, 1:10, 1:15, 1:20, and 1:25, i.e., 5 mg of Chrysophanol and 25 mg, 50 mg, 75 mg, 100 mg, and 125 mg of Pluronic
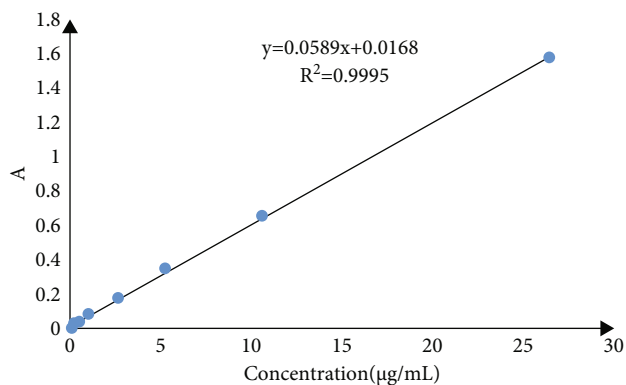


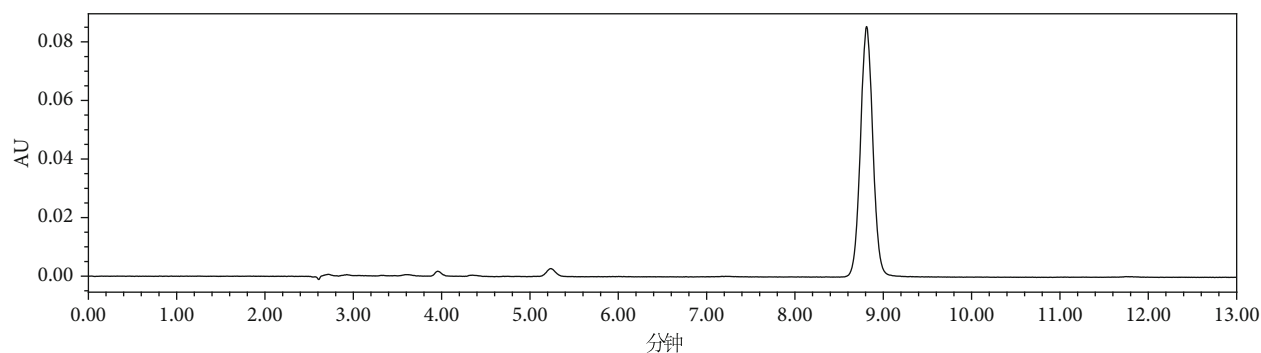FIGURE 12: The standard curve of in vitro dissolution.

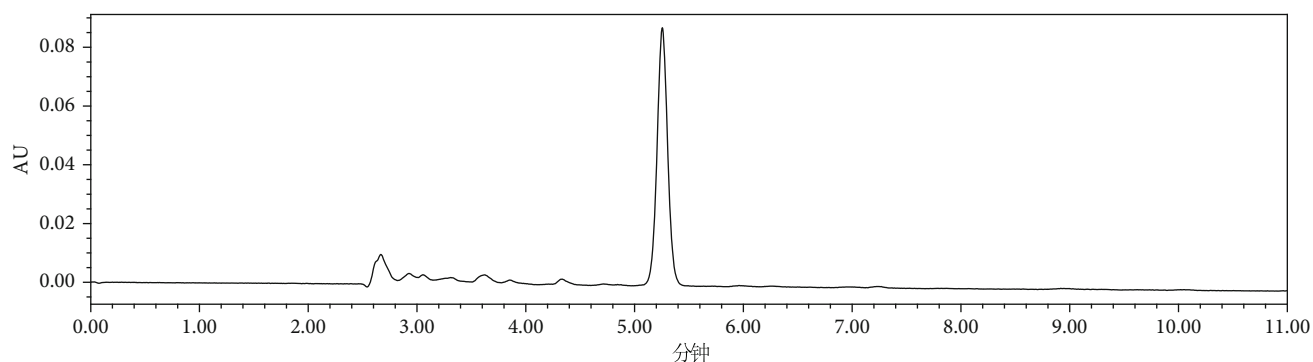FIGURE 13: The HPLC spectrum of Chrysophanol.



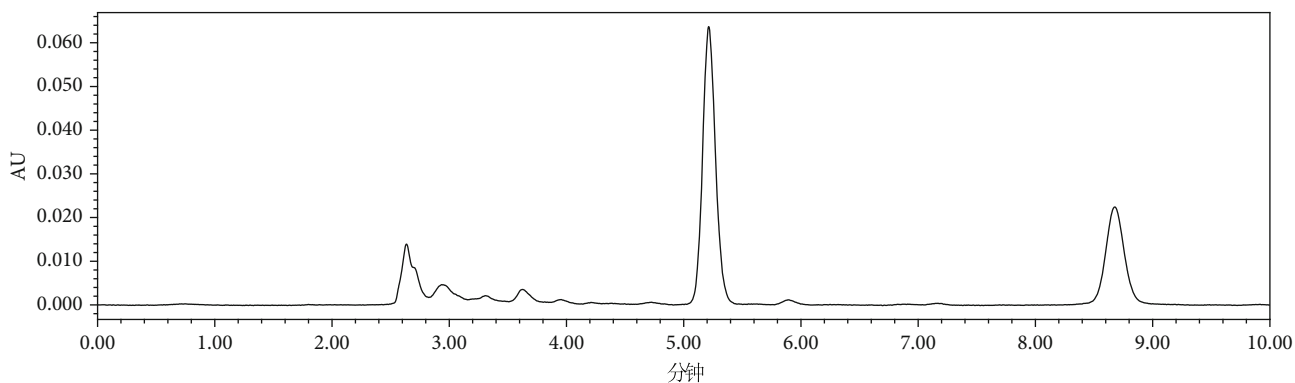FIGURE 14: The HPLC spectrum of blank rabbit plasma.



FIGURE 15: The HPLC spectrum of rabbit plasma after drug administration.

F127. The maximum encapsulation efficiency of $67.58 \pm 1.36\%$ was achieved when the ratio of drug amount to excipient amount was 1 : 15, i.e., 5 mg for Chrysophanol and 75 mg for Pluronic F127

(2) Chrysophanol-Pluronic F127 nanomicelles were prepared when the organic solvent (acetone) volume conditions were set to 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL. The maximum encapsulation efficiency was $67.00 \pm 0.98\%$ when the organic solvent volume was 8 mL

*4.2.2. Analysis of Variance and Model Fitting of Optimal Prescription by CCD Design Method.* The prescription of Chrysophanol-Pluronic F127 nanomicelles prepared by dial-ysis was optimized by CCD design method. The analysis of variance of the optimized prescription model by CCD design method is shown in Table 1. The results of significant differences of the models are shown in Table 2. The model fit is shown in Figures 6 and 7, and the fitted equation obtained is

$$r1 = 166.93629 + 16.86478A + 32.55582B - 0.169750AB$$
$$- 0.482675A^2 - 2.25797B^2 \left( R^2 = 0.9457 \right).$$

(2)

It can be seen that the model fits well and has a small error. The binomial model fit showed a significant difference, $P < 0.01$. Factor $A$ (the ratio of drug dose to excipient
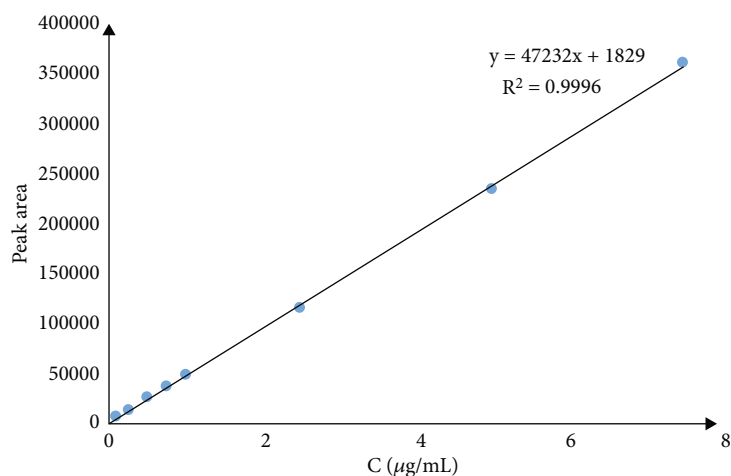
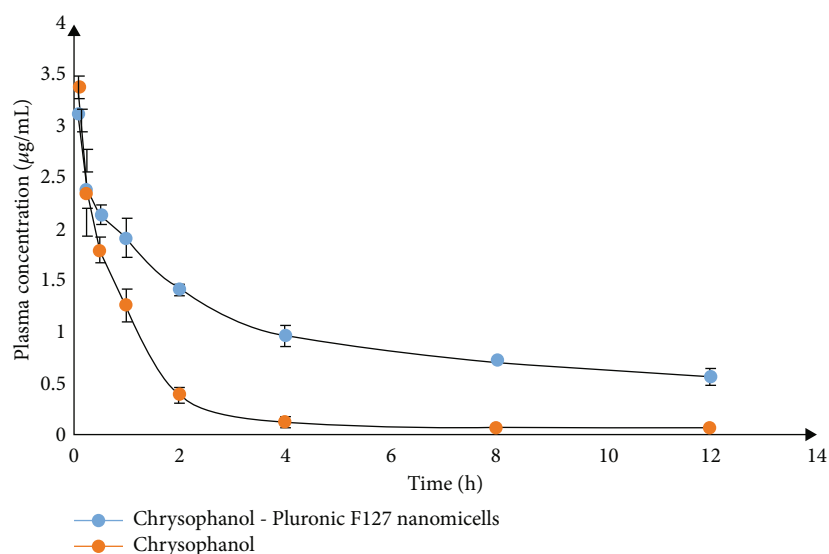FIGURE 16: The standard curve of Chrysophanol in rabbit plasma.



FIGURE 17: Plot of blood concentration changes for time over a 12 h period. The error bars represent the standard deviation of six independent sample measurements ($n = 6$).

dose) had a significant effect ($P < 0.01$). Factor $B$ (amount of organic solvent (acetone)) had a significant effect ($P < 0.01$), with the degree of effect being $B > A$. In the interaction term, $AB$ had no significant effect ($P > 0.05$). In the secondary term, A2 ($P < 0.01$) had a more significant effect and $B^2$ ($P < 0.01$) had a more significant effect.

4.2.3. Optimum Preparation Conditions. It was proved that the optimal prescription conditions were as follows: the ratio of drug dose to excipient dose is 1 : 16.309, and organic solvent volume is 6.595 mL. The encapsulation efficiency was $78.05 \pm 0.79\%$, RSD = 1.01%, RSD < 2%, and the deviation from the predicted value was small, indicating that the test fit was in line with the requirements. The results are shown in Table 3.

4.3. The Particle Size, Potential, and Morphology of Nanomicelles. The particle size of Chrysophanol-Pluronic F127 nanomicelles in water was 152.8 nm with a PDI of 0.225. The zeta potential was -23.9 mV, as shown in Figures 8 and 9. Under transmission electron microscopy, the Chrysophanol-Pluronic F127 nanomicelles appeared to be in a homogeneous black spherical shape, as shown in Figure 10.

4.4. In Vitro Release Studies. As shown in Figure 11, the in vitro release curve was established and the release of Chrysophanol-Pluronic F127 nanomicelles was significantly better than that of Chrysophanol. Standard curves for in vitro release were established, the fitted equation obtained is Equation (3), and the results are shown in Figure 12.

TABLE 4: The main pharmacokinetic parameters in rabbits.

| Pharmacokinetic parameters | Unit | Chrysophanol ($n = 6$, $x \pm s$) | Chrysophanol-Pluronic F127 nanomicelles ($n = 6$, $x \pm s$) |
| --- | --- | --- | --- |
| $t1/2_\alpha$/h | h | $0.308 \pm 0.207$ | $0.469 \pm 0.347$ |
| $t1/2_\beta$/h | h | $2.059 \pm 1.140$ | $7.715 \pm 2.035$ |
| V | (mg)/($\mu$g/ml) | $1.569 \pm 0.767$ | $2.099 \pm 0.358$ |
| AUC | $\mu$g/ml*h | $4.233 \pm 1.290$ | $12.186 \pm 0.603$ |
| CL | (mg)/($\mu$g/ml)/h | $1.716 \pm 0.359$ | $0.399 \pm 0.053$ |
| MRT | h | $1.762 \pm 0.831$ | $10.386 \pm 2.429$ |

$$y = 0.0589x + 0.0168 \left( R^2 = 0.9995 \right). \qquad (3)$$

### 4.5. In Vivo Pharmacokinetic Study in Rabbits

*4.5.1. Establishment of HPLC Standard Curve.* According to the chromatographic conditions under Section 3.4.1, the maximum absorption peak of Chrysophanol was measured at $8.7 \pm 0.3$ min, and the maximum absorption peak of blank rabbit plasma was measured at $5.2 \pm 0.2$ min, which did not interfere with each other, and the results are shown in Figures 13 and 14. The HPLC spectrum of rabbit plasma measured after administration is shown in Figure 15. The standard curve established was Equation (4), and the results are shown in Figure 16.

$$y = 47232x + 1829 \left( R^2 = 0.9996 \right). \qquad (4)$$

*4.5.2. Plasma Concentration-Time Profiles in Rabbits.* The time profiles of plasma concentration of Chrysophanol solution and Chrysophanol-Pluronic F127 nanomicelles solution injected intravenously at the ear margins of six rabbits, respectively, are shown in Figure 17.

*4.5.3. Analysis of Pharmacokinetic Parameters.* The model was embedded with the PK Solver 2.0 pharmacokinetic calculation program for Chrysophanol and Chrysophanol-Pluronic F127 nanomicelles plasma concentration-time data. The results showed that the variation of plasma concentration with time after intravenous injection of Chrysophanol and Chrysophanol-Pluronic F127 nanomicelles was consistent with the two-compartment model. The pharmacokinetic parameters were calculated on this basis, and the parameters showed that the distribution of Chrysophanol and Chrysophanol-Pluronic F127 nanomicelles in rabbits was basically the same, and the elimination time of Chrysophanol-Pluronic F127 nanomicelles was about three times longer than that of Chrysophanol, which greatly improved the residence time of the drug in rabbits, and the specific results are shown in Table 4.

## 5. Conclusions

In this study, Chrysophanol-Pluronic F127 nanomicelles were prepared by dialysis method, which is simple and easy to prepare under laboratory conditions and easy to adjust the preparation conditions. In order to avoid the influence of continuous variables in the experiment, a central composite design method different from the traditional experimental design was used in this study. This method is able to fit the corresponding surface model well and gives intuitive optimization regions to obtain the best preparation conditions. And the particle size potential and appearance morphology of the prepared Chrysophanol-Pluronic F127 nanomicelles were investigated by Malvern particle size meter and transmission electron microscope. It was found that the prepared nanomicelles have uniform particle size and good potential and appear as uniform black spheres under transmission electron microscopy. Through in vitro release experiments, it was demonstrated that the prepared nanomicelles could significantly improve the water solubility of Chrysophanol, and the in vitro release of Chrysophanol-Pluronic F127 nanomicelles was significantly higher than that of Chrysophanol. The treatment of the blood samples in the pharmacokinetic section, using ethyl acetate resolubilization, was more scientific than the traditional precipitation of proteins using methanol alone. The traditional method of protein precipitation using organic solvents such as methanol [26–28] does not take into account the possibility of drug precipitation with proteins or the possibility of complete removal of other components of the blood sample on the experimental results. The ethyl acetate resolubilization method in this study is more likely to avoid the loss of drug in the blood sample and the influence of other components in the blood sample. The results of pharmacokinetic study showed that the pharmacokinetic process of Chrysophanol in rabbits after intravenous injection at the ear margin was in accordance with the open two-compartment model, with rapid distribution and mainly elimination process, which belongs to the slow elimination class of drugs. After intravenous injection of Chrysophanol nanomicelles at the ear margin of rabbits, the pharmacokinetic process in rabbits was also consistent with the open two-compartment model, with faster distribution and no significant changes in Chrysophanol, but the elimination time of the drug was significantly longer, and the drug effect could exist in rabbits for a longer period of time. It is hoped that this study can provide a laboratory basis for the preparation of Chrysophanol-Pluronic F127 nanomicelles and pharmacokinetic studies and provide a reference for the clinical application of Chrysophanol.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no competing interest.

## Acknowledgments

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