Development and Optimization of Intravenous Puerarin Emulsions Formation by a Novel Complex-Phase Inversion-Homogenization Technology

Peng-Fei YUE,[a,b] Hai-Long YUAN,*[,a] Xian-Yi LI,[a] Ming YANG,[b] Wei-Feng ZHU,[b] and Xiao-He XIAO[a]

[a] *302 Hospital of PLA&PLA Institute of Chinese Materia Medica; Beijing 100039, China: and* [b] *Key Lab of Modern Preparation of TCM, Ministry of Education; Nanchang 330004, China.*

Received April 22, 2007; accepted August 31, 2007

To decrease the hemolysis side effect of puerarin, a novel approach of Complex-Phase Inversion-Homogenization (CPIH) Technology was established to prepare intravenous puerarin emulsions. Preparation of puerarin submicron emulsion were optimized by central composite design, and the physicochemical properties were evaluated. Puerarin phospholipid complexes prepared by puerarin and phospholipids at a ratio of 1 : 1.2. In order to improve the product quality, a central composite design was applied to optimize the critical process variables, such as emulsification time, stirring velocity and homogenization press, and the results were modeled statistically. Puerarin phospholipid complexs prepared were identified by fourier transform infrared spectrophotometry. The datas showed that the parameters had great effect on the response values. The particle size, span of dispersity and entrapment efficiency of puerarin emulsions prepared using the optimal parameters settings were 218.23 nm, 0.6284 and 87.32%, respectively. These meant that over 87% of the drug was located at the surfactant interface and oil droplet, the concentration of puerarin in aqueous was rarely. And the puerarin emulsion prepared by CPIH technology were sufficient stable for 90 d. The CPIH technology can be used as a general formulation principle for drugs which are slightly soluble in water and poorly soluble in oils.

Key words submicron emulsion; puerarin; complex-phase inversion-homogeneization; central composite design

Puerarin is naturally isoflavone *C*-glycoside which was isolated from *Pueraria lobota*. It is traditionally used to reduce febrile symptoms and decrease myocardial consumption of oxygen, and it also can increase coronary artery blood flow and improve microcirculation.[1—3] Due to poor oral bioavailability, the commercial product puerarin i.v. is widely used in clinical. It is used for treatment of coronary artery disease, ischemic heart disease, cerebrovascular disease, coronarism and cerebral angio spasm in clinic.[4,5] But the clinical efficacy of puerarin i.v. is limited by severe and acute toxic side effects, such as intravenous hemolysis. So it is very necessary to seek a novel delivery system for puerarin in order to reduce its side effects.[6,7]

Submicron emulsion, also referred to as lipid emulsions or lipid microspheres, is potentially interesting drug delivery system.[8] It can reduce drug hydrolysis and increase drug bioavailability.[9] By means of incorporating drug into the lipophilic core (of the oil droplets or in the core of micelles) or in the interface, direct contact of the drug with the body fluid and tissues can also be avoided in order to minimize the drug possible side effects. The reports about the ability of these systems to enhance the efficacy and to reduce side effects have appeared in the literature.[10] And there are some drug loaded emulsions for i.v. injection on the market such as amphotericin B,[11] prostaglandin E_1.[12]

Puerarin is slightly soluble in water (the aqueous solubility of about 4 mg/ml) and poorly soluble in oil.[13] So it would be very difficult to incorporate puerarin into oil phase of emulsion by the traditional homogenization method. The puerarin emulsions are not possibly prepared by applying the simple method which was to dissolve the drug in the oil in order to prepare the emulsion. The objectives of this study are: (a) puerarin emulsion was firstly prepared by novel complex-phase inversion-homogenization (CPIH) technology which was a technology basically combining drug phospholipid complex with the phase inversion-homogenization technology, and to investigate if it was possible to decrease the particle size, span of dispersity and to increase entrapment efficiency of puerarin emulsion; (b) to optimize preparative parameters which powerfully affect the characteristics of puerarin emulsion by central composite design; (c) to evaluate the physicochemical character of puerarin emulsion such as morphology and stability.

Experimental

Materials Puerarin was obtained from Xian-guochui Ltd., purity 99.5% (Xi-AN, China). Egg lecithin was purchased from Hua-qing-mei-hen Ltd. (Beijing, China); Purified soybean oil for parenteral use (Tieling BeiYa Pharmaceutical Co., Tieling, China). Synperonic F68 (BASF AG, Ludwigshafen, Germany), All other chemicals and reagents were of analytical or chromatographic grade.

Component of Preparation Formula Puerarin, 1 g; soybean lecithin, 1.2 g; soybean oil, 12 g; synperonic F68, 0.2 g; glycerol, 2.5 g; α-tocopherol, 300 mg; double distilled water, 90.0 g.

Preparation of Puerarin–Phospholipid Complexes The complex was prepared with puerarin and phospholipid at a weight ratio of 1 : 1.2. Weighed amount of puerarin and phospholipid were taken in a 100 ml round bottom flask and 60 ml of absolute alcohol was added. The mixture was refluxed at a temperature not exceeding 60 °C for 3 h. The resultant clear solution was evaporated to remove traces of solvents at 40 °C under vacuum.[14] Then the puerarin–phospholipid complexs were obtained.

Preparation of Puerarin Submicron Emulsion The lipid phase was prepared by dissolving puerarin–phospholipid complexes and α-tocopherol in soybean oil at 55 °C. The water phase was prepared by mixing water, synperonic F68 and glycerol at 55 °C. The lipid phase was stirred at 55 °C by high-speed stirrer, and the water phase was slowly influxed into the lipid phase by high-speed stirrer. This was very crucial step to control appropriate emulsification time and stirring velocity. The coarse emulsions were obtained. A fine emulsion was prepared by passing the coarse emulsion through high-press homogenizer at appropriate homogenization press (MPa) for 8 cycles at 25 °C (GYB40-20S, donghua homogenization apparatus, China). After adjusting the pH to 7 with 0.1 N sodium hydroxide solution, the emulsion was packed in 15 ml sterile glass vials under nitrogen. The

* To whom correspondence should be addressed. e-mail: ypfpharm@gmail.com

vials were sealed and the emulsions were sterilized by autoclaving at 121 °C for 15 min.

Fourier Transform Infrared Spectrophotometry (FT-IR) of Puerarin–Phospholipid Complexes Fourier transform infrared spectrophotometry (FT-IR Spectrometer, BRUKER IFS-55, Switzerland) was used to study the interaction between puerarin and phospholipids. The IR spectra of puerarin, phospholipids, the complex and a physical mixture of puerarin and phospholipids were obtained by the KBr method, respectively.

Particle Size Particle size was performed by laser diffractometry (LD) using a Sympatec Gmbh Nanophox (0119 P). The obtained distribution was a volume distribution, as characterization parameters the LD diameters 50, 90, 95 and 99% were calculated. For example, a diameter 90% (D_{90}) means that 90% of the volume of the particles is below the given size in nm. And the width of the particle size distribution was expressed as span of dispersity (SD), $SD=(D_{90}-D_{10})/D_{50}$.

Entrapment Efficiency (EE %) of Puerarin Emulsion Chromatographic column Spherisorb ODS C18 (250 mm×4.6 mm, 5 μm) was used for chromatographic separation. Mobile phase consisted of a mixture of methanol–H_2O (30 : 70, v/v) delivered at a flow rate of 1.0 ml/min. The injection volume was 20 μl. Detection was performed at 250 nm at room temperature.

The regression equation of puerarin was $A=11526C-8342.2$ ($r=0.9999$). The assay was linear in the concentration range 1.25—25 $\mu g\cdot ml^{-1}$. The percentage recoveries ranged from 98.72 to 101.80%, and the mean was 99.46%.

The emulsions with drugs were centrifuged at 23000×***g*** for 30 min (4 °C) in a Beckman Optima MAX ultracentrifuge (Beckman Coulter, Fullerton, CA, U.S.A.) in order to separate the incorporated drug and the non-incorporated drug. The drug was analyzed by HPLC for the non-incorporated drug concentration to determine the entrapment percentage.

The concentrations of puerarin in the emulsion (n_1) and free drug in the aqueous (the non-incorporated drug) (n_2) were assayed by HPLC after dilution with methanol. EE% could be achieved by the following equation: $EE\%=(n_1-n_2)/n_1\times100\%$.

Light Microscopy Analysis Light microscopy was performed using a BH-2 microscope (Chongqing, China). The magnification selected was 1500-fold, oil immersion applied. Emulsion was analyzed undiluted; typically 20 microscopic fields were analyzed under polarized light for the detection of remaining drug crystals.

Transmission Electron Microscopy (TEM) Transmission electron microscope was performed using a HD-600 transmission electron microscope (Hitachi, Japan). A drop of the resultant emulsions was placed onto a carbon-coated copper grid, leaving a thin liquid film. The films on the grid were negatively stained by immediately adding a drop of 2.5% (w/w) sodium phosphotungstate (pH 6.8), removing the excess staining solution with a filter paper, and followed by through air-dry. The stained films were then viewed on a transmission electron microscope and photographed.

Zeta Potential Measurement Zeta potential analyzer (Zetasizer Nano, MalvernInstruments, U.K.) were used to study zeta potential of emulsion. Puerarin emulsions were prepared according to the method described previously. The sample was diluted with distilled water until the appropriate concentration of particles was achieved, and the sample was measured to calculate zeta potential.

Evaluation of Stability Puerarin submicron emulsions were stored at room temperature. Particle sizes, span of dispersity, Zeta potential and entrapment efficiency were determined after 10, 20, 30, 60, 90 and 120 d to evaluate their stability.

Experimental Design of Central Composite Design To reduce the number of trials and attain the highest amount of information on product properties, the screening was planned applying a circumscribed central composite design.

The effects of preparative parameters were investigated on the puerarin emulsion properties, such as emulsification time, stirring velocity during preparing coarse emulsions and homogenization press. According to the principal of central composite design, the emulsification time (X_1, min), stirring velocity (X_2, rpm) and homogenization press (X_3, MPa) defined as independent values were evaluated on three response values (Table 1), and the mean diameter (Y_1, nm), span of dispersity (Y_2), entrapment efficiency (Y_3) were defined as response values in the mathmatical modeling, respectively. Each of the 20 formulations of a trial was produced three times in order to estimate the precision of emulsification time, stirring velocity and homogenization press (see Table 2).

Statistical Evaluation The results were evaluated using the program Statistica (Version 8.0, SAS, U.S.A.). Linear equations, Eq. 1 and second-order polynomial equations, Eq. 2 were fitted to the data, respectively.

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3 \quad (1)$$

Where Y was the response value, b_0 was the constant, and b_1, b_2, b_3 were the regression coefficients. X_1, X_2, X_3 represented the main effect, respectively.

Table 1. Factor Levels for the Experimental Design

Independent variables	Level				
	−1.682	−1	0	1	1.682
X_1 (min)	7	10	15	20	23
X_2 (rpm)	660	1000	1500	2000	2340
X_3 (MPa)	13	20	30	40	47

Table 2. Experimental Design Table (Columns 1—4) with Experimentally Determined Values of Different Dependent Variables (Columns 5—8)

No.	X_1 (min)	X_2 ($r\cdot min^{-1}$)	X_3 (MPa)	Mean diameter (Y_1/nm)	Span of dispersity (Y_2)	Entrapment efficiency (Y_3/%)	OD (Y_3)
1	−1	−1	−1	823.54±15.68	1.5499±0.0019	74.91±2.11	0
2	−1	−1	1	793.02±24.76	1.3542±0.0102	84.42±1.16	0.1138±0.0137
3	−1	1	−1	346.21±17.52	0.9689±0.0078	85.92±1.84	0.6797±0.0231
4	1	1	−1	336.76±16.91	0.9596±0.0216	85.12±2.11	0.3081±0.0164
5	−1	−1	1	494.81±21.43	0.8814±0.0098	78.15±2.43	0.1336±0.0225
6	1	−1	1	444.78±13.43	1.4301±0.0182	76.37±2.11	0.3225±0.0167
7	−1	1	1	479.69±13.86	0.6922±0.0181	60.64±2.16	0
8	1	1	1	501.43±17.76	0.6856±0.0085	72.76±1.15	0.6312±0.0098
9	−1.682	0	0	348.56±41.58	0.6892±0.0101	62.64±2.31	0
10	1.682	0	0	411.42±15.64	0.6804±0.0198	80.64±1.89	0.6544±0.0193
11	0	−1.682	0	514.74±14.92	1.302±0.0201	81.64±2.83	0.3866±0.0159
12	0	1.682	0	897.86±18.37	0.6746±0.0124	82.46±2.13	0
13	0	0	−1.682	794.76±34.31	0.9889±0.0346	86.43±2.32	0.2381±0.0214
14	0	0	1.682	434.63±23.45	0.6649±0.0167	69.64±1.37	0
15	0	0	0	241.36±21.75	0.6856±0.0126	84.43±1.24	0.8225±0.0156
16	0	0	0	222.13±14.92	0.6912±0.0098	85.02±1.61	0.8214±0.0184
17	0	0	0	247.36±13.64	0.6873±0.0079	84.65±2.07	0.8345±0.0114
18	0	0	0	234.67±14.67	0.6916±0.0136	84.66±1.51	0.8312±0.0098
19	0	0	0	242.41±23.71	0.6934±0.0146	84.53±1.67	0.8205±0.0134
20	0	0	0	237.48±18.91	0.6936±0.0143	84.64±1.34	0.8265±0.0089

Data was expressed by mean±S.D. (*n*=3).

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_4X_1^2+b_5X_2^2+b_6X_3^2 +b_7X_1X_2+b_8X_1X_3+b_9X_2X_3 \quad (2)$$

Where Y was the response value, b_0 was the constant, and b_1, b_2, b_3, b_4, b_5, b_6, b_7, b_8, b_9 were the regression coefficients, X_1, X_2, X_3 represents the main effect, respectively. X_1^2, X_2^2, X_3^2 were the quadratic effect and X_1X_2, X_2X_3, X_1X_3 were the interaction effect.

The selection of equations were based on the significant coefficients for the different fitting methods, and the equations were used to produce three dimensional response surface graphs by the optimal experimental conditions predicted. Overall desirability (OD) was calculated from the geometric mean of the three desirabilities of each formulation in order to evaluate all the response values.[15] The values of mean diameter, span of dispersity, entrapment efficiency can be evaluated by OD. Data of OD were fitted to a second-order polynomial equation, through which three dimensional response surface graphs were produced. Optimum experimental conditions on the emulsification time, stirring velocity and homogenization press were selected from the stationary point of the response surfaces.

Results and Discussion

Identification of Puerarin Phospholipids Complexs The most commonly preparation method of submicron emulsion with drug was to dissolve the drug in the oil phase. However, puerarin emulsion was not prepared by this method because the aqueous solubility of puerarin was about $4\,mg\cdot ml^{-1}$. We must reduce the solubility of puerarin in aqueous and locate it in the interfacial region of emulsions in order to increase the entrapment efficiency. In this study, we prepared puerarin phospholipids complexs as described in Li Y., Pan W.S. *et al.*[14] The infrared spectra of puerarin, phospholipids, their physical mixture and the complex are shown in Fig. 1. There was a significant difference between the spectrum of puerarin (Fig. 1A) and phospholipids (Fig. 1B). The spectrum of the physical mixture (Fig. 1D) was similar with one of the puerarin, but the spectrums peaks of phospholipids at $1740\,cm^{-1}$ and $1460\,cm^{-1}$ still presented, the peaks of phospholipids at $1228\,cm^{-1}$ and $1060\,cm^{-1}$ were masked by the wide peaks of puerarin at $1260\,cm^{-1}$, $1105\,cm^{-1}$, $1080\,cm^{-1}$ and $1040\,cm^{-1}$. The spectrum of the physical mixture showed an additive effect of puerarin and phospholipids. There was a significant difference between the physical mixture and the complexs (Fig. 1C), and the spectrum of the puerarin showed some difference with the spectrum of complex, in which the characteristic absorption peaks of puerarin had some change at $1630\,cm^{-1}$ and $1260\,cm^{-1}$. Moreover, no new peaks were observed in the mixture and complex. These observations suggested that the puerarin phospholipids complexs were formed by some weak physical interactions between puerarin and phospholipids. It was considered that puerarin and phospholipids should have some interaction, such as the combination of hydrogen bonds or van der Waals force.[14,16,17]

Optimization of Emulsion Preparation Central composite design (CCD) was a response surface design which provided information on direct effects, pairwise interaction effects and curvilinear variable effects and was widely used for formulation and process optimization in the field of pharmaceutics.[18,19] It was very efficient and flexible, providing much information on experiment variable effects and overall experimental error in a minimal number of required runs. Therefore, circumscribed CCD was a very suitable tool for process optimization of preparation in this study.

The effects of central composite design on the particle size, span of dispersity and entrapment efficiency were investigated. The datas were showed in Table 1.

The linear equations for datas were fitted as follows:

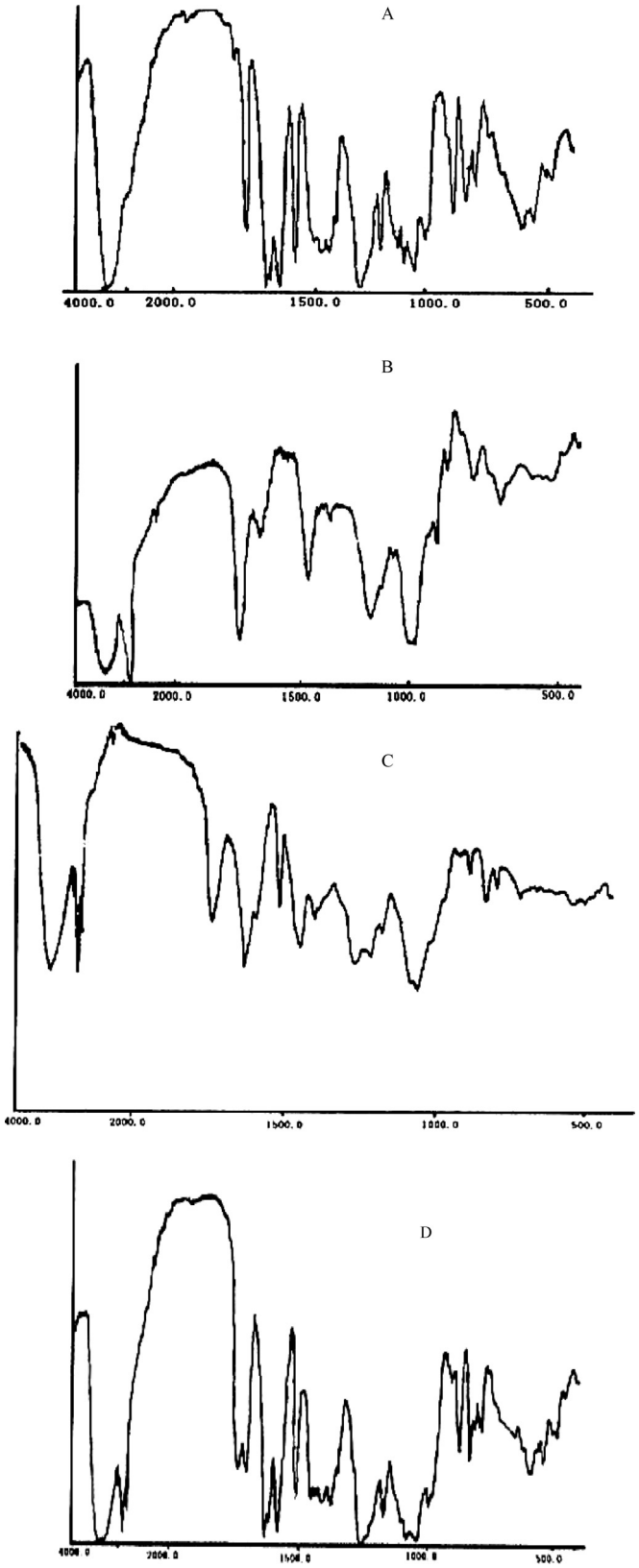

Fig. 1. Infrared Spectra of (A) Puerarin, (B) Phospholipids, (C) Complex, (D) Physical Mixture

$$Y_1=719.44-4.76X_1-3.57\times10^{-2}X_2-4.62X_3 \quad (r^2=0.0485, p<0.05)$$

$$Y_2=2.12-1.93\times10^{2}X_1-4.07\times10^{-4}X_2-1.08\times10^{-2}X_3 \quad (r^2=0.4072, p<0.05)$$

$$Y_3=85.87-0.191X_1-1.2\times10^{-3}X_2-6.43\times10^{-2}X_3 \quad (r^2=0.0212, p<0.05)$$

$$Y_4=0.059+1.59\times10^{-2}X_1+0.51\times10^{-4}X_2+1.16\times10^{-3}X_3 \quad (r^2=0.0441, p<0.05)$$

The second-order polynomial equations were fitted as follows:

$$Y_1=1281.72-64.83X_3-3.08\times10^{3}X_1X_2+2\times10^{-5}X_2^2+1.01X_3^2 \quad (r^2=0.9628, p<0.05) \quad (1)$$

$$Y_2=4.68-2.84\times10^{-3}X_2-0.098X_3+5.88\times10^{-7}X_2^2+2.2\times10^{-5}X_2X_3+0.91\times10^{-3}X_3^2 \quad (r^2=0.9429, p=0.05) \quad (2)$$

$$Y_3=18.26+7.22X_1+2.34\times10^{-2}X_2-0.16X_1^2-1.65\times10^{-3}X_1X_2-1.66\times10^{-3}X_3^2 \quad (r^2=0.8465, p<0.05) \quad (3)$$

$$Y_4=0.11+0.74\times10^{-2}X_1-0.52\times10^{-2}X_1^2+2.4\times10^{-5}X_1X_2+0.45\times10^{-2}X_1X_2-1.19\times10^{-7}X_2^2-1.13\times X_3^2 \quad (r^2=0.921, p<0.05) \quad (4)$$

It was noted that the coefficients of the second-order polynomial equation for mean diameter, span of dispersity, entrapment efficiency and over desirability (OD) were more higher than those of linear equation, so the second-order polynomial equation was more fit for data. The effects of mean diameter, span of dispersity and entrapment efficiency on response values were well evaluated by the second-order polynomial equation.

Figures 2 and 3 illustrated the relationship between the corresponding responses and factors. It was found that the extending stirring velocity and homogenization press caused a shift in the mean diameter. Generally speaking, the increasing stirring velocity and homogenization press can decrease the mean diameter and span of dispersity. The observed positive effect of stirring velocity and homogenization press was assumed to be a result of the fact that the particle size of emulsions was decreased because of input of high energy such stirring velocity and homogenization press. But on the other hand, the submicron emulsion system would be unstable because of the excessive consumption of water phase and the increase of temperature with consecutive input of higher energy, the mean diameter and span of dispersity would be increased again. Taken together with Eqs. 1, 2, so surface minimum of mean diameter and span of dispersity may be reached when the levels of X_2 and X_3 was 1500—2000 rpm and 20—30 min, respectively.

Figure 4 demonstrated that the changes in loading efficiency caused by the variation of the process variable emulsification time and homogenization press. It was observed that the maximum entrapment efficiency was near central region. It was the reason that the puerarin was entrapped into the interface membrane or oil phase to need adequate emulsification time, but the emulsion system would be unstable in order to breakdown with excessive prolonging of the emulsification time and increase of homogenization press. Taken together with Eq. 3, surface maximum would be reached when the levels of X_1 and X_3 are 15—20 min and 25—30 MPa, respectively.

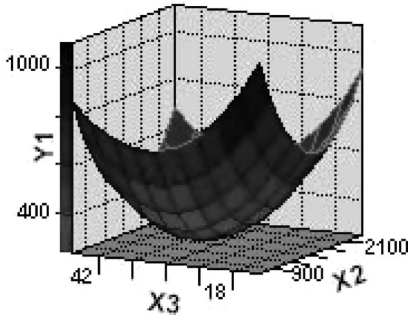

Fig. 2. Predicted Response Surface for Mean Diameter (Y_1) as a Function Stirring Velocity (X_2) and Homogenization Press (X_3), with Emulsification Time Equal to 15 min

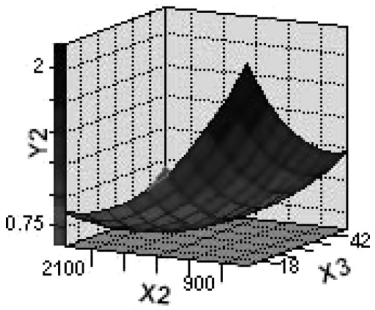

Fig. 3. Predicted Response Surface for Span of Dispersity (Y_2) as a Function Stirring Velocity (X_2) and Homogenization Press (X_3), with Emulsification Time Equal to 15 min

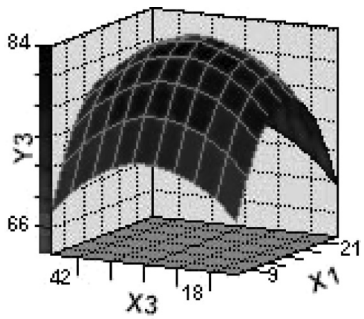

Fig. 4. Predicted Response Surface for Loading Efficiency (Y_3) as a Function Emulsification Time (X_1) and Homogenization Press (X_3), with Stirring Velocity Equal to 1500 rpm

Figures 5 and 6 demonstrated the effects of overall desirability (OD) on emulsification time, stirring velocity, homogenization press. Taken together with Eq. 4, it was noted that surface maximum would be reached when the levels of X_1, X_2 and X_3 was 14—16 min, 1600—2000 rpm and 30—36 MPa, respectively.

It was concluded that optimal range of emulsification time (X_1), stirring velocity (X_2), homogenization press (X_3) obtained from response surface was 14—16 min, 1600—2000 rpm and 30—36 MPa, respectively.

Validation of Model Optimization In order to evaluate

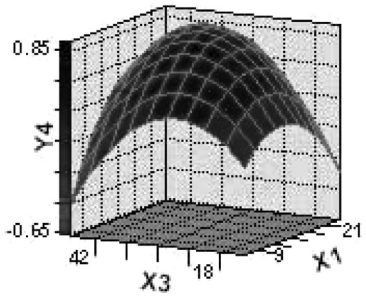

Fig. 5. Predicted Response Surface for OD (Y_4) as a Function Emulsification Time (X_1) and Homogenization Press (X_3), with Stirring Velocity Equal to 1500 rpm

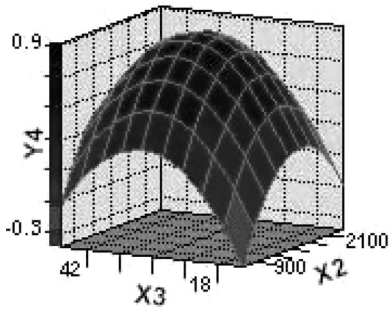

Fig. 6. Predicted Response Surface for OD (Y_4) as a Function Stirring Velocity (X_2) and Homogenization Press (X_3), with Emulsification Time Equal to 15 min

Table 3. Model-Predicted and Observed Values of Loading Efficiency, Mean Diameter and Span of Dispersity of Puerarin Intravenous Emulsion Prepared According to the Optimal Experimental Conditions (X_1=15, X_2=2000, X_3=30) (n=3)

Dependent variable	Predicted	Observed	Bias[a)]/%
Mean diameter (Y_1)	227.64	218.23±13.57	4.13
Span of dispersity (Y_2)	0.6223	0.6284±0.0082	−0.98
Entrapment efficiency (Y_3)	85.46	87.32±1.13	−2.18
OD (Y_4)	0.8664	0.8329±0.0097	3.35

a) Bias was calculated according to equation: bias/%=(predicted value − observed value)/predicted value ×100%.

the optimization capability of the models generated according to the results of the circumscribed central composite design, submicron emulsions were prepared using the optimal process variable settings that X_1, X_2 and X_3 were equal to 15 min, 2000 rpm and 30 MPa, respectively. The mean diameter, span of dispersity and entrapment efficiency obtained with predicted models were shown in Table 3. The results showed good agreement on preparation properties with theoretical predictions. The puerarin emulsions can be prepared successfully by CPIH.

It was reported that SolEmul technology was a preparation method of emulsion for the drug which is simultaneously poorly soluble in water and in organic media. The mixture was homogenized until the drug crystals were dissolved. The result of this process was that an ultra-fine emulsion was obtained with the drug located in the interfacial lecithin layer.[20] Puerarin is slightly soluble in water and poorly soluble in oil, we had studied that the entrapment efficiency of puerarin emulsion prepared by SolEmul technology was 56.48%, but the entrapment efficiency of puerarin emulsion prepared by

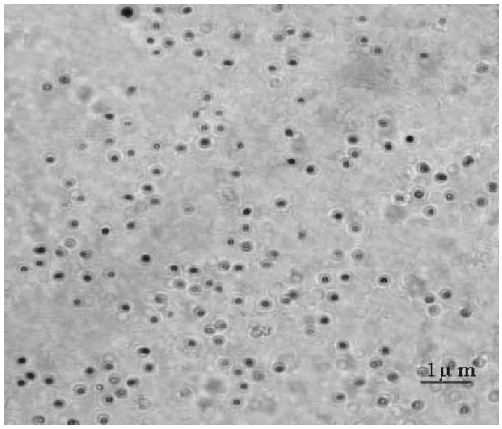

Fig. 7. Light Microscopy Analysis of Emulsion Load with 10 mg/ml Drug

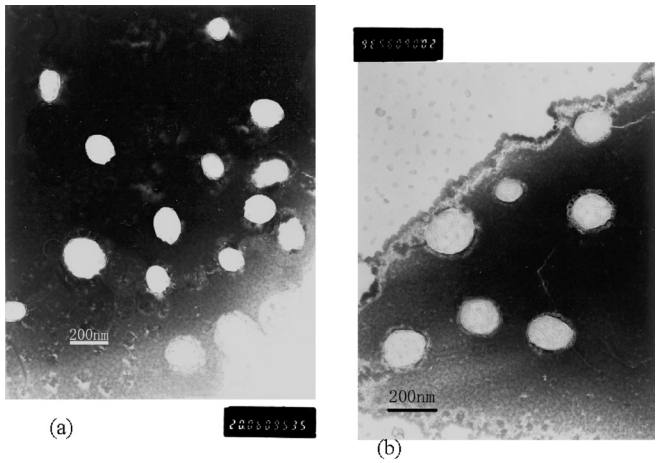

Fig. 8. TEM Graphs of the Blank Emulsions without Puerarin (a) and Puerarin-Loaded Emulsion (b)

CPIH technology was 87.32%. These showed that the entrapment efficiency was markedly increased. It was the reason that the phospholipids complexs could alter hydrophilicity and lipophilicity of puerarin,[14] it was easy to block the aqueous dissolution of puerarin and to increase the dissolution in oil. We thought that the CPIH technology could be used as a simple preparation method to incorporate drugs into parenteral emulsions, *i.e.* drugs which are slightly soluble in water, poorly soluble in oils and easily combined with phospholipids.

Light Microscopy Analysis It was difficult to identify non-incorporated drug particles in an ivory-white emulsion for laser diffractometry, and it could not differentiate between similarly sized droplets and drug crystals.[21] Accordingly, light microscopy was applied to examine puerarin emulsion with concentrations of 10 mg/ml, it was investigated whether there was the solid drug particles in the emulsion. The puerarin emulsion was not diluted to increase the probability of detecting the presence of even only a few solid drug particles. Figure 7 showed that no drug crystals were found in the emulsion.

Transmission Electron Microscopy (TEM) The electron microscopy micrographs of puerarin emulsion were shown in Fig. 8. The shape of emulsion droplets was spherical. And in comparison to the blank emulsion without loaded drug (Fig. 8a), the puerarin loaded emulsion showed a differ-

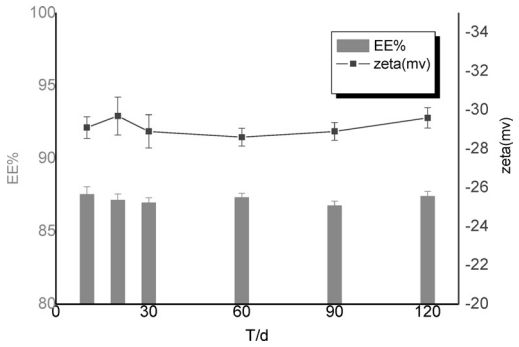

Fig. 9. Entrapment Efficiency (EE%) and Zeta Potential (mV) as a Function of Store Time (T) for Puerarin Intravenous Emulsion at Room Temperature

Data was expressed by mean±S.D. (n=3).

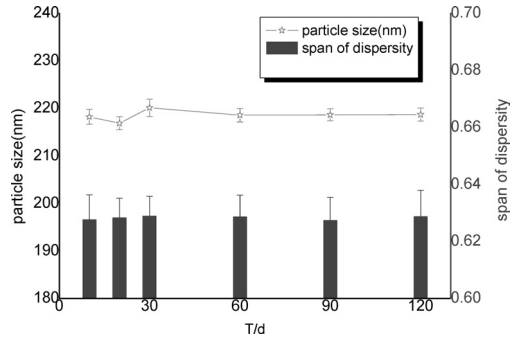

Fig. 10. Particle Size (nm) and Span of Dispersity as a Function of Store Time (T) for Puerarin Intravenous Emulsion at Room Temperature

Data was expressed by mean±S.D. (n=3).

ent appearance at the oil droplet surface (Fig. 8b). The change (Fig. 8) was attributed to incorporation of puerarin into the lecithin or oil droplets. It supported the hypothesis that puerarin was not only encapsulated in oil droplets but also distributed in the lecithin layer by specifically binding with lecithin in a ordered structure. The observed structures are being presently being investigated.

Evaluation of Stability The measurement of the zeta potential and particle size allow predictions about the storage stability of submicron emulsion. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion.[22] Figures 9 and 10 showed that particle sizes, span of dispersity, zeta potential and entrapment efficiency were very stable with slight change ($p>0.05$) after storaged 10, 20, 30, 60, 90 and 120 d. It was proved that the puerarin emulsions prepared by CPIH technology were sufficiently stable.

Conclusions

In this study, puerarin emulsions were successfully prepared by the novel complex-phase inversion-high press homogeneization technology, which was a technology basically combining drug phospholipid complex with the phase inversion-high press emulsification technology. It can increase the entrapment efficiency of drug in the emulsion by the phospholipid complex, and the particle size of emulsions prepared by the phase inversion-high press homogeneization technology is very small, the distribution range is very narrow. Puerarin phospholipid complexs were obtained according to the results of fourier transform infrared spectrophotometry. The puerarin phospholipid complexs was easy to solve in the oil in order to increase the entrapement efficiency of emulsion. The parameters of phase inversion-high press homogeneization technology were obtained by central composite design. The results showed that puerarin emulsions could be produced with a drug load as high as $10\,mg\cdot ml^{-1}$, and near 87% of the drug was located at oil phase, the entrapment efficiency of emulsion was very markedly increased, and the particle size and the distribution range of particle were completely acceptable. These showed a good agreement with the prediction of the models. The CPIH technology can be used as a general formulation principle for incorporating drugs into parenteral emulsions, *i.e.* drugs which are slightly soluble in water, poorly soluble in oils.

References

1) Liu Q., Lu Z., Wang L., *J. Tongji Med. Univ.*, **20**, 43—45 (2000).
2) Guerra M. C., Speroni E., Broccoli M., Cangini M., Pasini P., Minghetti A., Crespi-Perellino N., Mirasoli M., Cantelli-Forti G., Paolini M., *Life Sci.*, **67**, 2997—3006 (2000).
3) Lu X. R., Gao E., Xu L. Z., Li H. Z., Kang B., Chen W. N., Chen S. M., Chai X. S., *Chin. Med. J.*, **100**, 25—28 (1987).
4) Liao H. B., He Z. F., Wang G. C., Li H. J., Yue C. J., Chen X. F., *Sci. Technol. Food Ind.*, **24**, 81—82 (2003).
5) Fan L. L., Sun L. H., Li J., *Chin. Med. J.*, **105**, 7—11 (1992).
6) Zhang L. N., Shi H. Q., *Chinese J. Drug Application and Monitoring*, **3**, 1672 (2004).
7) Zhu Y. Z., Jiang B., Ma M. X., *Chinese J. New Drugs and Clinical Remedies*, **22**, 699 (2003).
8) Kawakami S., Yamashita F., Hasida M., *Adv. Drug Delivery Rev.*, **45**, 77—88 (2000).
9) Nasirideen S., *J. Clin. Pharm. Ther.*, **23**, 57—65 (1998).
10) Forster D., Washington C., Davis S. S., *J. Pharm. Pharmacol.*, **40**, 325—328 (1988).
11) Fukui H., Koike T., Saheki A., Sonoke S., Seki J., *Int. J. Pharmaceut.*, **265**, 37—45 (2003).
12) Teagarden D. L., Andersonb B. D., Petre W. J., *Adv. Drug Delivery Rev.*, **20**, 155—164 (1996).
13) Lee D. Y. W., Zhang W.-Y., R. Karnati V. V. R., *Tetrahedron Lett.*, **44**, 6857—6859 (2003).
14) Li Y., Pan W. S., Chen S. L., Yang D. J., *Chinese Pharmaceut. J.*, **41**, 1162—1167 (2006).
15) Wu W., Cui G. H., Lu B., *Chin. Pharm. J.*, **8**, 531—533 (2000).
16) Venema F. R., Weringa W. D., *J. Colloid Interface Sci.*, **125**, 484—500 (1988).
17) Lasonder E., Weringa W. D., *J. Colloid Interface Sci.*, **139**, 469—478 (1990).
18) Krogars K., Heinamaki J., Vesalahti J., Marvola M., Antikainen O., Yliruusi J., *Int. J. Pharmaceut.*, **199**, 187—194 (2000).
19) Dévay A., Mayer K., Pál S., Antal I., *J. Biochem. Biophys. Methods*, **69**, 197—205 (2006).
20) Akkara A., Mullerb R. H., *Eur. J. Pharm. Biopharm.*, **56**, 29—36 (2003).
21) Lixin W., Haibing H., Xing T., Ruiying S., Dawei C., *Int. J. Pharmaceut.*, **323**, 161—167 (2006).
22) Washington C., *Adv. Drug Delivery Rev.*, **20**, 131—145 (1996).