Optimized Preparation, Characterization and Biodistribution in Heart of Breviscapine Lipid Emulsion

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Breviscapine is a Traditional Chinese Medicine treating cardiovascular diseases by promoting blood circulation and removing blood stasis. The major active component of breviscapine has low aqueous solubility, poor chemical stability, short biological half-life and rapid elimination rate from the plasma. The use of a lipid emulsion formulation containing breviscapine might improve chemical stability, increase drug loading, exhibit sustained release profile. In the present study, we developed an optimized formulation and technological method for the preparation of sterile and stable breviscapine lipid emulsion (Bre-LE) for intravenous infusion. The average particle size, polydispersity index, zeta potential, stability constant (K_s) value and content of final product were (225.3±8.8) nm, 0.221±0.020, (−29.6±1.5) mV, (24.3±2.9) % and (94.5±0.6) % respectively (n=3). The results of *in vitro* release experiment suggest that lipid emulsion as breviscapine carrier showed a desirable sustained release profile. Dilution stability and long-term stability were also researched in the present paper. The results show the carrier could protect drug from degradation after dilution by phosphate buffered saline and fetal calf serum. And Bre-LE was stable for up to 6 months at room temperature storage condition. The biodistribution of drug in heart of mice increased dramatically after encapsulation into lipid emulsion which was beneficial to heart disease therapy.

Key words breviscapine; lipid emulsion; scutellarin; optimized preparation; biodistribution

Cardiovascular diseases are the world's largest killers. Traditional Chinese Medicine has special advantages in treating cardiovascular diseases, such as low toxicity and well-known therapeutic effects. Breviscapine is a flavone glucuronide extracted from a Chinese herb *Erigeron breviscapinus* (VANT.) HAND.-MAZZ.[1] It is widely used in the treatment of angina pectoris, coronary heart disease, cerebral infarction and its sequelae. It contains mainly scutellarin (4′,5,6-tetrahydroxyflavone-7-*O*-glucuronide, primary active ingredient) (for chemical structure see Fig. 1) and little apigenin-7-*O*-glucuronide. Scutellarin, the major active component of breviscapine, has low aqueous solubility, poor chemical stability, short biological half-life and rapid elimination rate from the plasma.[2,3] Breviscapine and its preparation (*Injectio Breviscapine*) were listed in the Pharmacon Criteria (Chinese Traditional Patent Medicine). *Injectio Breviscapine* is aqueous solution of water-soluble salt of breviscapine.

Nanoparticles-based drug delivery systems have considerable potential for treatment of cardiovascular diseases. The important technological advantages of nanoparticles used as drug carriers are high efficiency targeting to infracted myocardium,[1,4—6] high carrier capacity,[7] feasibility of incorporation of both hydrophilic and hydrophobic substances.[8,9] These properties of nanoparticles enable improvement of drug bioavailability[10] and reduction of the dosing frequency. Among drug carriers, lipid emulsion as drug carriers combine advantages of polymeric nanoparticles, liposomes and micelles. Furthermore lipid emulsion can be produced on large industrial scale and sterilized by autoclaving but avoiding drug leakage from carriers like liposomes.[11,12] The use of a lipid emulsion formulation containing breviscapine might improve the chemical stability, increase drug loading, decrease irritation on the surrounding tissue as well as control and modify its pharmacokinetics and tissue distribution. In the present study, we developed an optimized formulation and technological method for the preparation of sterile and stable breviscapine lipid emulsion (Bre-LE) for intravenous infusion. The characterization and biodistribution in heart of Bre-LE was also researched.

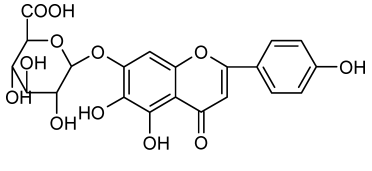

Fig. 1. Chemical Structure of Scutellarin

Experimental

Materials Breviscapine was provided by Jiangsu Chia-tai Tianqing Pharmaceutical Co., Ltd. (Jiangsu, China). Scutellarin standard (purity >98%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Lipoid E80 (egg yolk lecithin with 80—85% of phosphatidylcholine) was purchased from Lipoid GmbH (D-Ludwigshafen, Germany). Poloxamer 188 was purchased from BASF (China) Co., Ltd. (Shanghai, China). Triton X-100 was purchased from Sigma. *Injectio Breviscapine*, which is an injection solution of scutellarin (20 mg/5 ml) was produced by Gejiu Bio-Medicine Industry Ltd. (Yunnan, China). Other chemicals used were of analytical grade.

Preparation of Bre-LE Bre-LE was prepared as follows. The oil phase was prepared by dissolving scutellarin (0.04%) in the mixture of required amounts of Lipoid E80, oleic acid, vitamin E (abbreviated V_E) (0.6%) and soybean oil using a sonicator (B5200S-DT Sonicator, Branson Ultrasonics Co., Ltd., Shanghai, China). To make the aqueous phase, required amounts of Poloxamer 188 dissolved in a mixture of glycerol (2.25%) and double distilled water. A preemulsion was prepared by mixing the oil phase and the aqueous phase with a constant speed stirrer (XHF-1 Stirrer, Shanghai Xinda BioChem Instrument Co., Ltd., Shanghai, China). Final emulsification was completed by passing the preemulsion through a homogenizer (EmulsiFlex-05 High Pressure Homogenizer, Avestin Inc., Canada) and autoclaved. The formulation and technological parameters of breviscapine lipid emulsion were optimized on the basis of univariate analysis and orthogonal experiment design as shown in Table 1. Composite grade method was used to evaluate the preparation. The composite grade index (S) was calculated accord-

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Table 1. Factors and Levels of Orthogonal Experiments Design

Levels	Factors			
	A (Lipoid E80, %)	B (Poloxamer 188, %)	C (Oleic acid, %)	D (Pressure, psi)
1	0.8	1.8	0.6	12500
2	1.0	2.0	0.8	15000
3	1.2	2.2	1.0	17500
4	1.4	2.4	1.2	20000

ing to the following equations:

$$S=(S_1+S_2+S_3+S_4)/4$$

$$S_1=[(D_{max}-D)/(D_{max}-D_{min})]\times 100\%$$

where D is the particle size; D_{max} is the maximum particle size among all results of orthogonal experiment; D_{min} is the minimum particle size among all results of orthogonal experiment;

$$S_2=[(PI_{max}-PI)/(PI_{max}-PI_{min})]\times 100\%$$

$$S_3=[(|\zeta|-|\zeta|_{min})/(|\zeta|_{max}-|\zeta|_{min})]\times 100\%$$

$$S_4=[(K_{s\,max}-K_s)/(K_{s\,max}-K_{s\,min})]\times 100\%$$

where PI, $|\zeta|$ and K_s are polydispersity index, absolute value of zeta potential and stability constant, respectively. Analogously, "max" and "min" presents the maximum and minimum of each evaluation index, respectively.

Measurement of Size Distribution and Zeta Potential Particle size and width of the distribution (polydispersity index, *PI*) were determined by photon correlation spectroscopy (MasterSizer 3000, Malvern Instruments Co., Worcestershire, U.K.). Data were analyzed in terms of intensity, volume and number distributions and reported as *z*-average diameter. The zeta potential (ζ) was obtained by measuring the electrophoretic mobility (Malvern Zetasizer 3000, Malvern Instruments Co., Worcestershire, U.K.). The laser in this equipment was operated at 532 nm using a 90° angle between incident and scattered beams. All samples were diluted 100 times by 2.25% glycerin at 25 °C.

Measurement of Stability Constant (K_s) Stability constant was determined by centrifugation-spectrophotometry method.[13] In details, 5 μl sample was centrifuged at 4000 rpm for 15 min. 0.1 ml supernatant before and after centrifugation was diluted to 10 ml by water. Then the absorption of diluted sample was determined by spectrophotometry with detection wavelength at 500 nm and water blank as a reference. The stability constant $K_s(\%)=(A_0-A)/A_0\times 100\%$. A_0 was the absorption of dilution suspension of supernatant before centrifugation. A was the absorption of dilution suspension of supernatant after centrifugation.

***In Vitro* Drug Release** *In vitro* release in phosphate buffered saline (abbreviated PBS) is at pH 7.4 and fresh rat plasma were evaluated using a dialysis bag diffusion technique. The mixture of 1 ml Bre-LE and 1 ml PBS or fresh rat plasma were placed in dialysis bag (8000—10000 molecular weight (MW) cutoff, 25 mm, Sigma) and immersed in 150 ml normal saline stirred continuously at 37 °C. Aliquots of 2 ml dissolution medium were removed and the same volume of fresh dissolution medium was added periodically. Twenty microliters aliquots were measured by HPLC.

Determination of Scutellarin in Normal Saline and Bre-LE by Reverse Phase (RP)-HPLC Assay The concentration of scutellarin in Bre-LE was measured by RP-HPLC with dissolving Bre-LE in 10% Triton X-100 in ethanol for breaking emulsion. A 20 μl aliquot of the supernatant fluid of broken Bre-LE or scutellarin sample in normal saline was injected into HPLC for assay. The chromatographic system[14] consisted of a Waters 510 HPLC pump and a Waters 486 Absorbance UV detector (Waters Corp., Milford, MA, U.S.A.). The wavelength of this detector was set to 335 nm. The HPLC system was controlled by a computer employing the Millennium 2010 ChemStation software. The analytical column was a reverse phase Hypersil C_{18} column (250×4.6 mm, 5 μm particle size; Dalian Elite Analytical Instrument Co., Ltd., Dalian, China) maintained in a column oven (Timberline Instruments, Boulder, CO, U.S.A.). The mobile phase was composed of methanol–water–glacial acetic acid (40 : 60 : 1). Elution was performed isocratically at 40 °C at a flow-rate of 1.0 ml/min.

Dilution Stability and Long-Term Stability To investigate the ability of carriers to protect drug from degradation after dilution by PBS (pH 7.4) and PBS containing 10% fetal calf serum, Bre-LE without V_E (Bre-LE-1) was used. The same procedures were applied for preparing Bre-LE-1 as it for Bre-LE except for no V_E added to the oil phase. Breviscapine solution (Bre-Sol) was also prepared as control just before used. In details, 40 mg of breviscapine dissolved in 5 ml double distilled water with 17 mg $NaHCO_3$ as pH adjuster. After drug dissolved, this solution was diluted with normal saline to 100 ml. Dilution stability experiment as follows: Bre-LE-1 and Bre-Sol were diluted 10-fold with PBS or 10% fetal calf serum at 37 °C. The samples were withdrawn at appropriate intervals and prepared as reference.[14] The scutellarin remained in the diluted samples was determined by HPLC.

Long-term stability of Bre-LE was evaluated after storage at room temperature for up to 6 months. The particle size, polydispersity index, zeta potential, stability constant K_s and content of scutellarin in Bre-LE were determined as a function of the storage time. The content of scutellarin was determined by HPLC. The particle size, polydispersity index, zeta potential and stability constant K_s were measured as described previously.

Biodistribution of Bre-LE in Heart Kunming mice with an average weight of 20 g were used in this study. The mice were divided into two groups of three to five animals. Breviscapine lipid emulsion and *Injectio Breviscapine* were injected intravenously into the tail vein of the mice (25 mg/kg). Animals were sacrificed under ether anesthesia at indicated intervals after administration. Heart was removed, weighed and homogenized (10%, w/v) in a solution of 1% sodium bisulfate in physiological saline. All samples were immediately frozen at −20 °C until analysis. A 200 μl aliquot of methanol was added to 100 μl aliquot of homogenate. Then, the mixed samples shaked on a SW-80A vortex shaker (Shanghai Medical University Instrument Plant, Shanghai, China) and were centrifugated at 10000 ***g*** for 10 min at 4 °C (Refrigerated Centrifuge 3K30, Sigma, German). A 20 μl aliquot of the supernatant fluid was injected into HPLC for assay. The chromatographic system consisted of a Waters 510 HPLC pump and a Waters 486 Absorbance UV detector (Waters Corp., Milford, MA, U.S.A.). The wavelength of this detector was set to 335 nm. The HPLC system was controlled by a computer employing the Millennium 2010 ChemStation software. The analytical column was a reverse phase Hypersil C_{18} column (250×4.6 mm, 5 μm particle size; Dalian Elite Analytical Instrument Co., Ltd., Dalian, China) maintained in a column oven (Timberline Instruments, Boulder, CO, U.S.A.). The mobile phase was composed of methanol–water–glacial acetic acid (40 : 60 : 1). Elution was performed isocratically at 40 °C at a flow-rate of 1.0 ml/min.

Results and Discussion

Univariate Analysis for Formulation and Technological Method To study effects of technological method on properties of preemulsion and final emulsion during the preparation, the lipid emulsion were prepared with the fixed composition: breviscapine (0.04%), Lipoid E80 (1.2%), V_E (0.06%), oleic acid (1.0%), soybean oil (10%), poloxamer 188 (2.0%) and glycerol (2.25%). The results of mixing temperature, striring time and stirring rate on preemulsion preparation are shown in Figs. 2—4. Smaller particle size, polydispersity index and K_s value were achieved at higher temperature of mixing. The content of scutellarin decreased to below 98% beyond 80 °C. Thus, the optimized temperature of mixing was chosen as 80 °C. It was also seen that particle size and polydispersity index decrease first, then increase with stirring time and rate increasing. It was due to the dispersed oily droplets coagulated under high shearing force provided by constant speed stirrer during oil phase dispersion simultaneously.[13] Another reason was the bubbles formed in the process of high speed stir could weaken shearing force and then increase the collision of oily droplets.[13] Thus, the optimized stirring time and rate of the preemulsion mixture were chosen as 60 s and 8000 rpm.

Homogenization could lower the interfacial tension between oil and water phase resulting in a more stable emulsion. The results of cycle number and operating pressure of

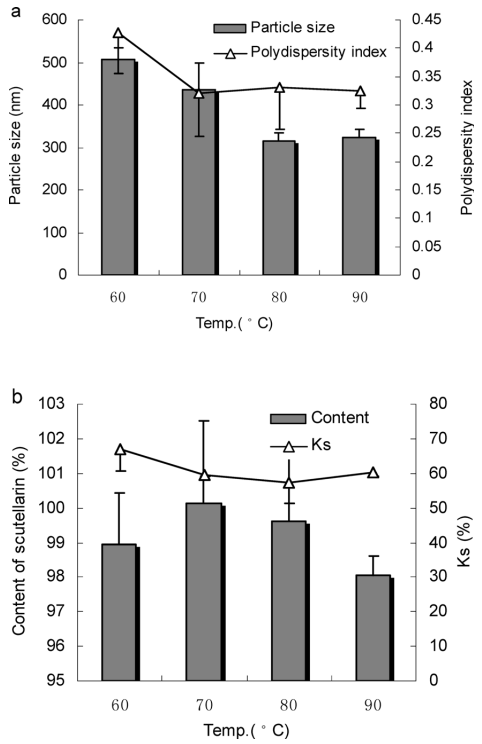

Fig. 2. Particle Size, Polydispersity Index (a) and Content of Scutellarin, K_s Value (b) of Preemulsion as a Function of Temperature of Mixing (8000 rpm, 60 s, Mean±S.D., n=3)

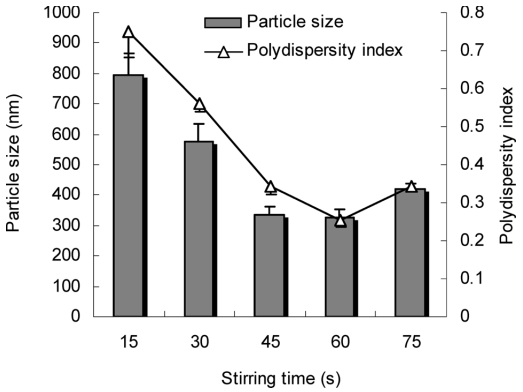

Fig. 3. Particle Size and Polydispersity Index of Preemulsion as a Function of Stirring Time (8000 rpm, Mean±S.D., n=3)

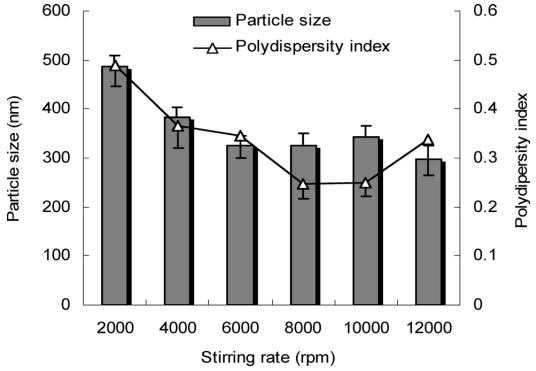

Fig. 4. Particle Size and Polydispersity Index of Preemulsion as a Function of Stirring Rate (60 s, Mean±S.D., n=3)

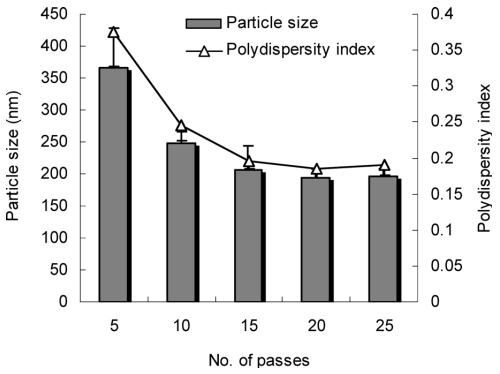

Fig. 5. Particle Size and Polydispersity Index of Final Emulsion as a Function of Number of Cycles through the Homogenizer (20000 psi, Mean±S.D., n=3)

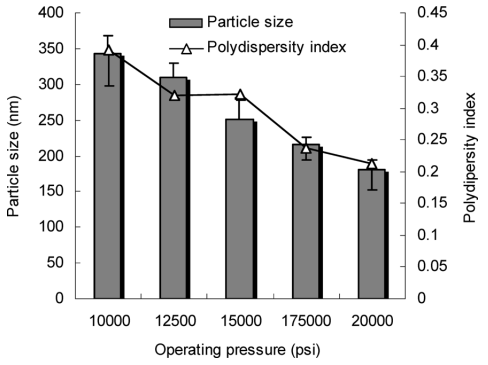

Fig. 6. Particle Size and Polydispersity Index of Final Emulsion as a Function of Operating Pressure (20 Cycles, Mean±S.D., n=3)

homogenization on final emulsion are shown in Figs. 5 and 6. It was seen that smaller particle size and polydispersity index were observed at higher number of cycles and operating pressure of homogenization. But there was no significant difference between 20 and 25 passes. Thus, the optimized cycle number was chosen as 20 passes.

Figure 7 summarizes the effects of sterilization method on particle size, polydispersity index, zeta potential, K_s value and scutellarin assay. As the results show, larger particle size was found at higher temperature of sterilization due to the combination of oily droplets. The physical stability of breviscapine lipid emulsion were improved and the particle was uniform which could be concluded by smaller polydispersity index and K_s value, higher absolute value of zeta potential achieved at higher temperature of sterilization. But significant loss (more than 5%) in scutellarin content was observed when the heat temperature increased beyond 100 °C. Thus, the optimized sterilization method was chosen as 100 °C for 30 min. Although the heat-treatment may be insufficient, we could take measures to remove microorganism for compensation, such as: some excipients in the formulation adopted were intravascular administration grade, the preparation was passed through a 0.22 μm sterilizing-grade membrane filter performed under aseptic conditions. And the preparation has no pyrogen examined by limulus test.

Oleic acid concentration was an important factor on the physical stability of breviscapine lipid emulsion. Results show that the properties of breviscapine lipid emulsion changed with different oleic acid concentration before and after steril-

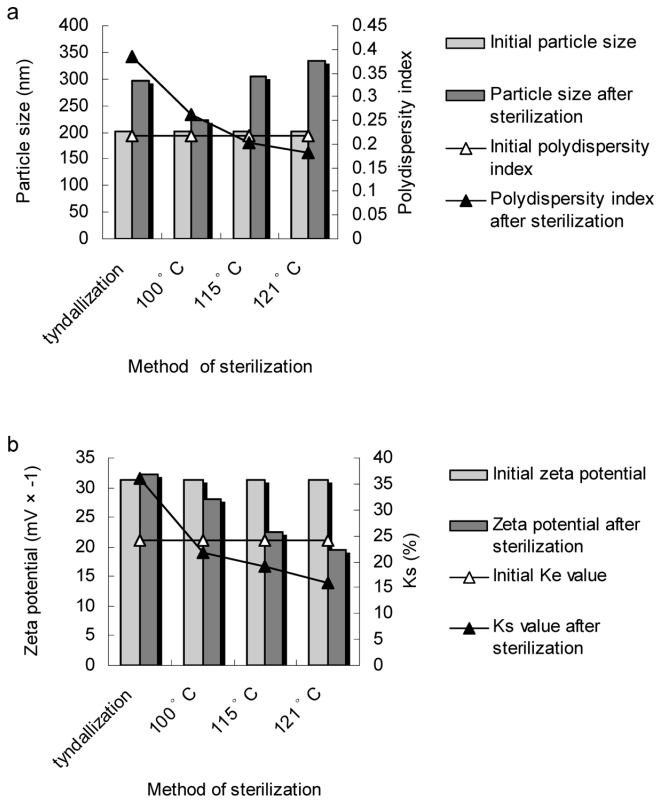

Fig. 7. Particle Size and Polydispersity Index (a), Zeta Potential and K_s Value (b), Content of Scutellarin (c) of Bre-LE before and after Sterilization (S.D. <8%, n=3)

Tyndallization: heat to 80 °C for 1 h and then at 25 °C for 24 h, cycle three times. Other sterilization method was 100 °C for 30 min, 115 °C for 30 min and 121 °C for 15 min.

ization (Fig. 8). There was no data shown with formulation composition of 0% and 20% oleic acid after sterilization, because drug precipitation (0% oleic acid) and emulsion broken (20% oleic acid) were observed during heating process, respectively. Breviscapine solubility in oil phase was improved with oleic acid added. Otherwise, overdose of oleic acid could not be emulsified by Lipoid E80 and Poloxamer 188. Oleic acid could not only increase breviscapine solubility, but also attribute to increase the absolute value of zeta potential and then improve physical stability properties conferred to the emulsion due to strengthening of the molecular interactions occurring between phospholipid and Poloxamer emulsifiers in the presence of an ionized form of oleic acid at

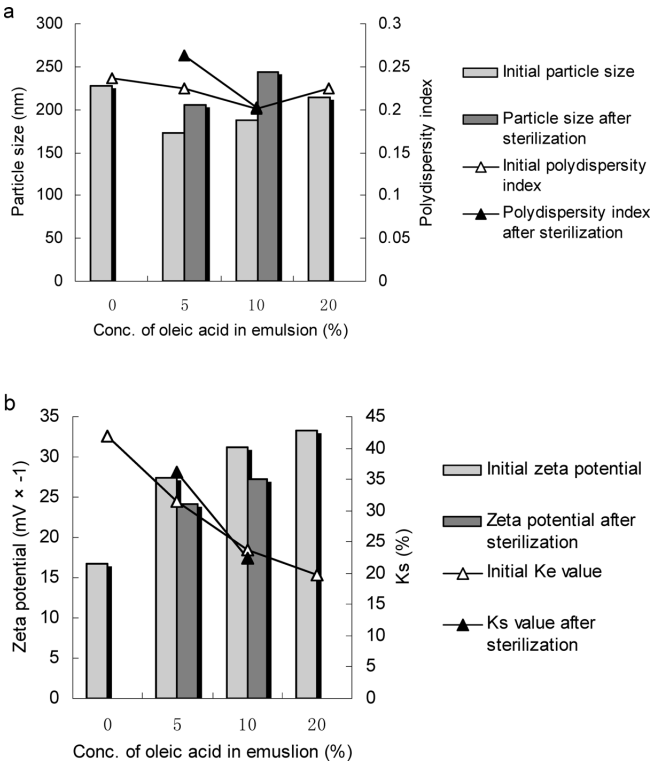

Fig. 8. The Particle Size and Polydispersity Index (a), Zeta Potential and K_s Value (b) of Bre-LE as a Function of Oleic Acid Concentration in Emulsion before and after Sterilization (121 °C, 15 min, S.D. <8%, n=3)

the o/w interface of the emulsified oily droplets.[15]

Orthogonal Experiment Design for Formulation and Technological Method Based on univariate analysis above and experience found in preparation, orthogonal experiment design was performed to further optimize the formulation and technological parameter of homogenization. The emulsion was prepared with some of fixed parameters: temperature of mixing (80 °C), stirring time and rate (60 s and 8000 rpm) for preemulsion preparation, number of cycles for final emulsion homogenization (20 passes) and autoclave method parameters for sterilization (100 °C for 30 min). The composition scale: breviscapine (0.04%), Lipoid E80 (0.8—1.4%), V_E (0.06%), oleic acid (0.6—1.2%), soybean oil (8.8—9.4%), poloxamer 188 (1.8—2.4%), glycerol (2.25%). The results of orthogonal experiment design were given in Table 2. The sequence of effect on composite grade to evaluate the preparation was: D>A>C>B. The optimum preparation parameters were $A_3B_2C_4D_4$, that is Lipoid E80=1.2%, poloxamer 188=2.0%, oleic acid=1.2%, pressure=20000 psi.

Optimum Formulation and Technological Method The oil phase was prepared by dissolving breviscapine (0.04%) in the mixture of Lipoid E80 (1.2%), V_E (0.06%), oleic acid (1.2%) and soybean oil (8.8%) using a sonicator. To make the aqueous phase, poloxamer 188 (2.0%) was dissolved in a mixture of glycerol (2.25%) and double distilled water. A preemulsion was prepared by mixing the oil phase and the aqueous phase with a constant speed stirrer at 8000 rpm for 60 s. Final emulsification was completed by passing the preemulsion through a homogenizer at 20000 psi for twenty times. Sterilization was performed by heating at 100 °C for 30 min.

Characterization of Bre-LE Follow the optimum

Table 2. The Results of Orthogonal Design for Optimization of Bre-LE Formulation and Technological Method

Test No.	Factors				Score				
	A	B	C	D	S_1	S_2	S_3	S_4	S
1	0.8	1.8	0.6	12500	9.9	94.1	74.6	0	43.8
2	0.8	2.0	0.8	15000	19.6	76.4	49.2	68.2	52.4
3	0.8	2.2	1.0	17500	30.7	77.9	63.8	69.1	59.0
4	0.8	2.4	1.2	20000	51.6	94.8	83.1	71.1	72.8
5	1.0	1.8	0.8	17500	45.8	100.0	50.8	70.2	64.6
6	1.0	2.0	0.6	20000	96.0	53.1	38.4	83.5	63.4
7	1.0	2.2	1.2	12500	23.6	0	96.0	91.2	51.6
8	1.0	2.4	1.0	15000	64.7	64.7	64.4	63.0	55.5
9	1.2	1.8	1.0	20000	67.7	77.3	70.1	69.1	71.0
10	1.2	2.0	1.2	17500	68.5	86.3	72.3	100.0	78.7
11	1.2	2.2	0.6	15000	45.2	90.7	0	93.0	55.2
12	1.2	2.4	0.8	12500	16.9	64.3	41.8	83.4	50.8
13	1.4	1.8	1.2	15000	37.4	32.7	100.0	32.6	49.0
14	1.4	2.0	1.0	12500	0	32.4	84.7	66.2	45.8
15	1.4	2.2	0.8	20000	100.0	81.4	75.1	37.4	73.5
16	1.4	2.4	0.6	17500	67.7	84.8	45.2	49.3	58.7
K_1/4	58.2	58.1	57.7	48.6					
K_2/4	61.1	62.2	61.3	54.6					
K_3/4	65.4	60.9	58.6	67.7					
K_4/4	57.9	61.4	65.1	71.9					
R	7.5	4.0	7.4	23.2					

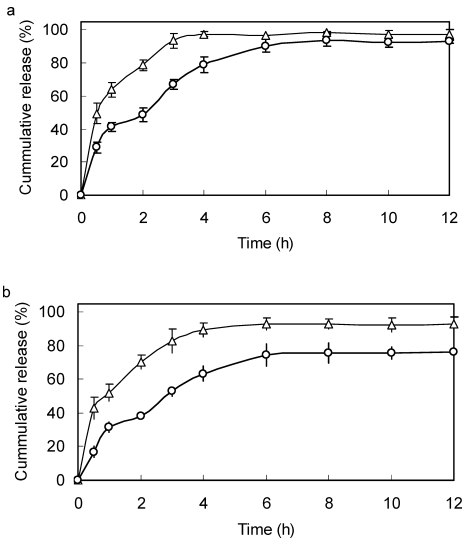

Fig. 9. *In Vitro* Release of Scutellarin from Bre-LE (○) When Incubated with PBS (a) and Rat Plasma (b) at 37 °C (Mean±S.D., n=3)

Release of *Injectio Breviscapine* (△) was also presented as a control.

preparation found above, the average particle size, polydispersity index, zeta potential, K_s value and content of final product were (225.3±8.8) nm, 0.221±0.020, (−29.6±1.5) mV, (24.3±2.9)% and (94.5±0.6)% respectively (n=3). Bre-LE is safe for intravenous administration because the size distribution was narrow and the particle size was smaller than 1 μm. The results of zeta potential and K_s value suggest Bre-LE is stable.

***In Vitro* Release** The release of scutellarin was studied in both PBS and fresh rat plasma. As shown in Fig. 9, free scutellarin in control samples (*Injectio Breviscapine*) diffused freely across the membrane of dialysis bag, and in fewer than 6 h of dialysis, nearly 100% of the free drug had

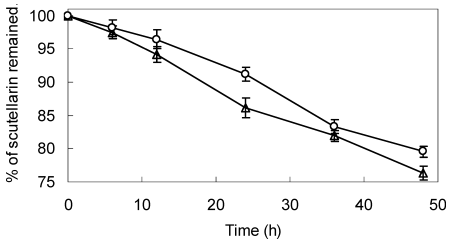

Fig. 10. Degradation of Bre-Sol (△) and Bre-LE-1 (○) Diluted with PBS at 37 °C (Mean±S.D., n=3)

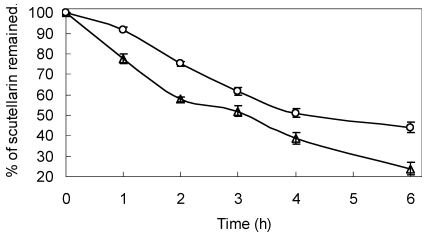

Fig. 11. Degradation of Bre-Sol (△) and Bre-LE-1 (○) Diluted with Fetal Calf Serum at 37 °C (Mean±S.D., n=3)

crossed the membrane to the release medium of both PBS and fresh rat plasma. However, less than 80% of drug was released from Bre-LE after 12 h in fresh rat plasma. These results suggest that lipid emulsion as breviscapine carrier showed a desirable sustained release profile. The drug leaked from oil phase to aqueous phase in the preparation could be negligible because the saturated solubility of scutellarin was very low and the amount of drug leaked was lower than or equal to the saturated solubility regarding no precipitation observed in the preparation. Therefore the leakage could not influence the results of release test.

Dilution Stability and Long-Term Stability Drug was diluted when it was injected into body. The dilution stability experiment was designed to simulate the chemical stability *in vivo*. The degradation mechanism of scutellarin was mainly oxidation. The lipid emulsion could protect drug in oil phase from meeting oxygen in the air because the aqueous phase was a barrier between the air and oil phase. We need to investigate the protective ability of lipid emulsion carriers itself, not the ability of stabilizer to protect drug from degradation. Therefore Bre-LE without V_E was used for removing the disturbance of stabilizer on the results. The results of dilution stability in PBS and 10% fetal calf serum are shown in Figs. 10 and 11. The results show there was a nearly linear degradation of scutellarin in both PBS and 10% fetal calf serum. The first-order rate constant of Bre-Sol and Bre-LE-1 were 0.0060 and 0.0050 in PBS, 0.2339 and 0.1484 in fetal calf serum, respectively. The results show the carrier could protect drug from degradation after dilution by PBS and fetal calf serum. The dilution stability is very important to drug with poor chemical stability. Because drug degradation happened after injection is one of the key factors inducing shorter half-life in plasma.

The long-term stability data for Bre-LE under storage at room temperature is summarized in Table 3. The particle size, polydispersity index and K_s value increased slowly. And absolute value of zeta potential and content of scutellarin decreased gently. Based on the data, Bre-LE was physically

Table 3. Long-Term Stability of Bre-LE under Storage at Room Temperature (Mean±S.D., $n=3$)

Storage time (month)	Particle (nm)	Polydispersity index	Zeta potential (mV)	K_s (%)	Content of scutellarin (%)
0	225.3±8.8	0.221±0.020	−29.6±1.5	24.3±2.9	94.5±0.6
1	241.5±4.1	0.242±0.011	−26.2±2.0	26.0±1.7	94.2±0.7
3	242.8±10.9	0.245±0.009	−22.2±1.0	26.9±2.9	93.6±0.9
6	251.0±15.5	0.251±0.012	−17.4±2.7	27.4±0.9	92.5±0.3

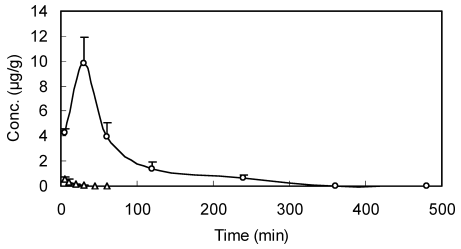

Fig. 12. The Concentration–Time Curves of Scutellarin in Heart of Mice Given *Injectio Breviscapine* (△) and Breviscapine Lipid Emulsion (○) after Intravenous Administration

Each point represents the mean±S.D. of three to five mice.

and chemically stable for 6 months at room temperature.

Biodistribution of Bre-LE in Heart The mean concentration *vs*. time profile of scutellarin following intravenous administration of *Injectio Breviscapine* and breviscapine lipid emulsion is showed in Fig. 12. The area under concentration (*AUC*) was calculated by logarithmic thrapezoidal method. The maximum tissue concentration (C_{max}) was obtained directly from the individual concentration–time profiles. Mean retention time between time 0 and the infinite ($MRT_{0\to\infty}$) was determined using a log-linear trapezoidal method 3P97 (Mathematic Pharmacological Committee, Chinese Pharmacological Society, China). The tissue distribution advantages in heart of lipid emulsion carrier were very obvious: the *AUC* values of Bre-LE (623.26 μg·min/ml) were much higher than that of *Injectio Breviscapine* (5.39 μg·min/ml). And the C_{max} of Bre-LE (9.84 μg/g at 30 min after administration) increased more than 18-fold *versus Injectio Breviscapine* (0.54 μg/g at 5 min after administration). The $MRT_{0\to\infty}$ of Bre-LE and *Injectio Breviscapine* were 62.62 and 8.17 min, respectively. The $MRT_{0\to\infty}$ of breviscapine increased more than 7-fold after encapsulation into lipid emulsion. It was possibly because breviscapine was a chemically unstable drug and easily degrade *in vivo*. Lipid emulsion, as a drug carrier, could protect breviscapine from degradation and therefore produced a significant change in the heart. Increasing amount and prolonging retention time of drug in heart were all beneficial to heart disease therapy.

Conclusion

We prepared a lipid emulsion of Traditional Chinese Medicine—breviscapine with its process easily produced on large industrial scale and sterilized by autoclaving. Bre-LE optimizedly prepared in this paper could protect scutellarin from degradation, exhibited sustained release, and was stable for up to 6 months at room temperature storage condition. Based on the results of biodistribution in heart, higher cardioprotective activity of Bre-LE than that of *Injectio Breviscapine* in further studies on pharmacodynamics may be expected.

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