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To improve bioavailability of pueraria flavones (PF), a self-microemulsifying drug delivery system (SMEDDS) dropping pills composed of PF, Crodamol GTCC, Maisine 35-1, Cremophor RH 40, 1,2-propylene glycol and polyethylene glycol 6000 (PEG6000) was developed. Particle size, zeta potential, morphology and in vitro drug release were investigated, respectively. Pharmacokinetics, bioavailability of PF-SMEDDS dropping pills and commercial Yufeng ningxin dropping pills were also evaluated and compared in rats. Puerarin treated as the representative component of PF was analyzed. Dynamic light scattering showed the ability of PF-SMEDDS dropping pills to form a nanoemulsion droplet size in aqueous media. The type of media showed no significant effects on the release rate of PF. PF-SMEDDS dropping pills were able to improve the in vitro release rate of PF, and the in vitro release of these dropping pills was significantly faster than that of Yufeng ningxin dropping pills. There was a dramatic difference between the mean value of $t_{1/2}$, peak concentration ($C_{\text{max}}$), the area of concentration–time curve from 0 to 6 h ($AUC_{0-6h}$) of PF-SMEDDS dropping pills and that of commercial Yufeng ningxin dropping pills. A pharmacokinetic study showed that the bioavailability of PF was greatly enhanced by PF-SMEDDS dropping pills. The value of $C_{\text{max}}$ and relative bioavailability of PF-SMEDDS dropping pills were dramatically improved by an average of 1.69- and 2.36-fold compared with that of Yufeng ningxin dropping pills after gavage administration, respectively. It was concluded that bioavailability of PF was greatly improved and that PF-SMEDDS dropping pills might be an encouraging strategy to enhance the oral bioavailability of PF.

Key words self-microemulsion; pueraria flavone (PF); dropping pill; release in vitro; relative bioavailability

Radix Puerariae, the dried root of the leguminous plant pueraria lobata (Willd.) Ohwi, is a traditional Chinese herb medicine. Pueraria flavones (PF), major active ingredients extract from pueraria, are mixtures of several active ingredients that include puerarin, daidzin, daidzein, puerarin-7-xiloside and so on. Puerarin is the most abundant and major active ingredient. PF were found effective clinically to treat a variety of cardiovascular diseases, including coronary heart disease, angina pectoris and hypertension. It was also effective in treating arrhythmia, migraines, sudden deafness and menopause, etc. 3, 4

However, the effectiveness of PF as cardiovascular disease remedy is restricted by its poor solubility, short elimination half-life and rapid first-pass metabolism after oral administration. Only 15.91% of PF are absorbed from the gastrointestinal tract after oral administration. 4 The commercial formulations of PF include dropping pills, tablets and injection. Only 3.95% of puerarin in Yufeng ningxin tablets is absorbed compared with puerarin injection. 5, 6 Yufeng ningxin dropping pills are a formulation of PF obtained from pueraria lobata and are marketed in China. In order to improve the bioavailability and dissolution of PF, various strategies have been employed, such as incorporating into dropping pills, preparing sustained-release tablets 7 and self-microemulsifying drug delivery system. 8

Recently, great attention has been focused on self-microemulsifying drug delivery system (SMEDDS), 9-11 benefitting from the success of cyclosporine formulations–Neoral. 12 SMEDDS is an effective pharmaceutical technology in improving the oral bioavailability of poorly water-soluble drugs bioavailability. SMEDDS is a mixture composed of drugs (poor solubility ones), lipids, surfactants and cosurfactants. SMEDDS forms oil-in-water (O/W) microemulsion with a particle diameter of less than 100 nm in aqueous media or the gastric and/or intestinal fluids under the condition of gentle agitation. The spontaneous formation of microemulsion advantageously presents the drug in a dissolution media, and the smaller particle diameter of SMEDDS provides a large interfacial surface area allowing them to be readily absorbed in the gastrointestinal tract.

Most of the SMEDDS formulations are encapsulated in gelatin capsules. However, this delivery system has some limitations, such as stability, and the interaction between the fillings and the capsule shell. To solve these problems, some strategies have been attempted to convert liquid SMEDDS into solid SMEDDS. Microcrystalline cellulose and lactose were added into liquid SMEDDS as solid carriers to produce self-emulsifying pellets by wet granulation. 12 Solid SMEDDS granules were prepared by spray-drying method with colloidal silica or dextran as solid carriers. 13 Polyvinyl alcohol or hydroxypropyl-β-cyclodextrin were used as carriers to form a solid SMEDDS microcapsule. 14, 15 SMEDDS tablets were developed by spray-drying through using mannitol as a carrier. 16 PF-SMEDDS dropping pills were prepared in our previous study. An orthogonal experimental design L9(34) was presented to optimize the technological preparation conditions of SMEDDS dropping pills. The optimal formulation of PF-SMEDDS dropping pills was selected with the mass ratio of 1:3.5 between liquid SMEDDS and polyethylene glycol 6000.
In the present study, the phase behavior of PF-SMEDDS was studied. Zeta potential, droplet size, morphology and release rate in vitro were chosen to evaluate the characterization of solid PF-SMEDDS dropping pills. Pharmacokinetic parameters, bioavailability of PF-SMEDDS dropping pills were measured and compared with that of Yufengningxin dropping pills after administration in rats.

**MATERIALS AND METHODS**

Pueraria flavones were purchased from Xi’an SaiBang Pharmaceuticals and Technology Co., Ltd. (China, purity: 66.70%). Standard puerarin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (No. 110752-200912; Beijing, China, purity: 99.10%). Crodamol GTCC, Maisine 35-1 and Labrasol were obtained from Gattefosse (France). Cremophor RH 40 and Solutol HS 15 were provided by BASF (Germany). Heparin sodium injection was purchased from Tianjin Biochemistry Pharmaceuticals Co., Ltd. (Tianjin, China). HPLC-grade methanol and acetonitrile were purchased from Fisher Scientific (U.S.A.). Yufengningxin dropping pills were obtained from Tengtang Technologies Co., Ltd. (Beijing, China). PEG6000, phosphotungstic acid and methyl silicon oil were supplied by Sinopharm Chemical Regent Co., Ltd. (Beijing, China). Methanol, 1,2-propylene glycol and carboxymethyl cellulose sodium salt were provided by Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Membrane filters were purchased from Tianjin Jinteng Experiment Equipment Co., Ltd. (Tianjin, China). Distilled water was supplied by Milli-Q (Germany).

**Pseudo-ternary Phase Diagram Study**

The pseudo-ternary phase diagrams of mixtures (oils, surfactants, cosurfactants and water) were constructed using a water titration method. In the present study, Cremophor RH 40 and 1,2-propylene glycol were selected as emulsifier and co-emulsifier, respectively. Oil and a specific S<sub>mix</sub> ratio (surfactant:cosurfactant, w/w) were carefully mixed in diverse weight ratios from 1:9 to 9:1 (w/w). Each sample was gradually diluted with distilled water and the visual appearance was noted. The mixture was observed by transmission electron microscope (TEM) (JEM-2010, Japan). Each experiment was prepared by the same procedure as zeta potential measurements. All data were expressed as the mean value ± standard deviation (S.D.).

**Drug Release in Vitro**

According to the apparatus of dissolution test in Chinese Pharmacopoeia Edition, paddle method was used to determine the drug release profile using 900mL distilled water as a dissolution medium. The temperature was set to 37°C and paddle revolution speed to 75 rpm. An aliquot of 5 mL was collected at intervals of 0, 2, 5, 10, 20, 40, 60 min and promptly replaced with an equal volume of fresh 37±0.5°C dissolution media. The samples were filtered through a membrane filter (0.45µm). The concentration of puerarin was determined by TU-1901 ultraviolet spectrophotometer (Beijing Purkinje General Co., Ltd., China) at a wavelength of 250 nm. All samples were carried out in triplicate. All data were expressed as the mean value ± S.D.

**Analysis of Puerarin in Plasma by Reverse Phase High Performance Liquid Chromatography (RP-HPLC)**

As puerarin is the major active component of PF, pharmacokinetic and bioavailability study of PF is always based on the determination of plasma puerarin. In this study, the concentration of puerarin in rat plasma (200 µL) was measured by extraction in 1mL of methanol. The samples were filtered through a 0.45 µm membrane filter. An aliquot of 20 µL was measured by HPLC. The LC-20AT HPLC (Shimadzu, Japan) system consisted of a LC-20AT pump and SPD-20A UV detector controlled by Lab-solution software. The analysis was carried out using an Diamonsil 5 µm C<sub>18</sub> column (4.6×250mm; Dikma, China) and guarded with a refillable pre-column (C<sub>18</sub>, 4.6×10mm; Dikma) at a column temperature of 25°C. The mobile phase was composed of methanol and distilled water at a volume ratio of 30:70. The flow rate was adjusted to 1.0mL/min and the wavelength was monitored at 250nm. All samples were carried out in triplicate.

**Oral Bioavailability Studies**

The purpose of this study was to compare pharmacokinetics parameters and bioavailability of PF-SMEDDS dropping pills (test sample) with those of Yufengningxin dropping pills (reference sample). Oral bioavailability assessment was performed in parallel groups of six rats. The suspension of the samples was prepared by plac-
ing the dropping pills into a cup with carboxymethyl cellulose sodium salt solution.

Experiments were conducted according to the Guidelines for Care and Use of Laboratory Animals. Male Sprague-Dawley rats (300 ± 25 g) were obtained from the Laboratory Animal Center of Jilin University (Changchun, China). All animals were fasted for 12 h with free access to water before the experiment. Twelve male rats were randomly divided into two groups. All of the groups received either PF-SMEDDS dropping pills suspension or Yufengningxin dropping pills suspension at an equivalent dose to 27.80 mg/kg of puerarin via gavage administration. Plasma samples (0.5 mL) were withdrawn from the caudal vein and placed in heparinized tubes at 0, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240 and 360 min after oral administration. Blood samples were separated from heparinized blood after centrifugation at 10000 rpm for 5 min using a centrifuge 5415C (Eppendorf, Germany) and stored at −20°C until analysis.

Pharmacokinetic data were analyzed using the DAS 2.1 pharmacokinetic software (program by Chinese Pharmacological Society, China). The area of concentration–time curve was calculated using the following formula:

\[ AUC = \int_0^{\infty} C(t) \, dt \]

where \( AUC \) is the area under the concentration–time curve, \( C(t) \) is the concentration at time \( t \), and \( t \) is the time after administration.

RESULTS AND DISCUSSION

**Pseudo-ternary Phase Diagrams** PF had the highest solubility in Maisine 35-1 of the oils, Cremophor RH-40 among the selected emulsifiers and 1,2-propanediol in the co-emulsifiers. The maximum self-microemulsion region was obtained when the emulsifier and co-emulsifier were mixed at a mass ratio of 3:2 (Fig. 1). The oil mixture of Crodamol GTCC and Maisine 35-1 showed an increased effect on the self-microemulsion region and the largest area of self-microemulsion was obtained at the blending mass ratio of 1:1 (Fig. 2d). The optimized formulation of liquid PF-SMEDDS was consisted of Crodamol GTCC, Maisine 35-1, Cremophor RH 40, and 1,2-propanediol at a ratio of 5:5:9:6 (w/w) with 17.65% drug loads (Table 1).

**Preparation of SMEDDS Dropping Pills** An orthogonal experimental design was a better way to qualitatively analyze the correlations between relevant factors and different levels. An orthogonal experimental design with four factors and at three levels L_9(3^4) was used to optimize the technological preparation conditions of SMEDDS dropping pills. The mass ratio between SMEDDS and the PEG6000 of dropping pills, dropping temperature and dropping distance were tested in our previous study. The effect factors of the dropping process were listed as follows: dropping temperature > dropping distance > the ratio of liquid PF-SMEDDS and PEG6000. Based on the orthogonal experimental data, the ratio of liquid PF-SMEDDS and PEG6000 (1:3.5), dropping temperature (80°C), dropping distance (10 cm) and cooler base (8°C) were selected as an optimized conditions to prepare SMEDDS dropping pills.

**Zeta Potential Analysis** Zeta potential is a vital parameter for SMEDDS which indicates either the electrostatic repulsion or congregation of the oily droplets. The electrostatic conflict forces of the SMEDDS droplets are critical factors for ensuring the stability of the systems. Increased electrostatic repulsive forces between the droplets might prevent coalescence of the microemulsion droplets from forming large oily globules. However, decreasing electrostatic repulsive forces can result in phase separation. A small difference was observed among liquid blank SMEDDS, liquid PF-SMEDDS and PF-SMEDDS dropping pills. Zeta potential of liquid blank SMEDDS and liquid PF-SMEDDS was (−3.78 ± 0.12) mV and (−4.11 ± 0.17) mV, respectively. Furthermore, the zeta potential of PF-SMEDDS dropping pills was (−6.33 ± 0.21) mV.

**Droplet Size Determination** Droplet size is one of the most important parameters of SMEDDS that affects the rate of drug release and drug stability. Smaller droplets have a greater interfacial area that significantly enhances the rate of drug release and facilitate lymphatic transport.

Droplet sizes of liquid blank SMEDDS, liquid PF-SMEDDS and PF-SMEDDS dropping pills were presented in Fig. 3 and Table 2. Liquid blank SMEDDS, liquid PF-SMEDDS and PF-SMEDDS dropping pills were presented with monomodal droplet size distribution (Fig. 3). The results (Fig. 3) showed that particle diameter displayed an increasing trend from liquid blank SMEDDS to liquid PF-SMEDDS or from liquid PF-SMEDDS to PF-SMEDDS dropping pills (17.84 ± 0.14 nm < 19.23 ± 0.15 nm < 34.32 ± 0.21 nm). The particle diameters of liquid blank SMEDDS, liquid PF-SMEDDS and PF-SMEDDS dropping pills were (17.84 ± 0.14) nm, (19.23 ± 0.15) nm and (34.32 ± 0.21) nm respectively in Table 2. Mean Polydispersity index (PDI) value of PF-SMEDDS dropping pills was higher than that of liquid blank SMEDDS and liquid PF-SMEDDS. The increased particle diameter and mean PDI value of the PF-SMEDDS dropping pills might result from the effect of PEG6000. It was reported that the average particle diameters of solid SMEDDS formulation were dependent on the solid carriers. According to the literature, PEG6000 could increase the viscosity of water pools of microemulsions which might increase droplet size of the PF-SMEDDS dropping pills.

In order to study dilution behavior of SMEDDS, the effect
of aqueous media dilution volume on particle size was evaluated. It showed that dilution volume within the investigated range had little effect on droplet size (Table 3) and self-microemulsifying behavior. Moreover, these PF-SMEDDS dropping pills were still transparent after dilution with water. The results had shown the ability of PF-SMEDDS dropping pill to form microemulsion droplet size.

Morphology Analysis Microemulsion would be produced by redispersed PF-SMEDDS dropping pills with deionized water. Therefore, it was particularly important to investigate the structural properties of the microemulsion. The morphol-

Table 1. Index of Various Oil/Smixed Ratio

<table>
<thead>
<tr>
<th>No.</th>
<th>Oil/Smixed ratio (w/w)</th>
<th>Drug loads (% w/w)</th>
<th>Self-emulsifying time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:9</td>
<td>19.26</td>
<td>&lt;300</td>
</tr>
<tr>
<td>2</td>
<td>2:8</td>
<td>18.61</td>
<td>&lt;180</td>
</tr>
<tr>
<td>3</td>
<td>3:7</td>
<td>17.93</td>
<td>&lt;150</td>
</tr>
<tr>
<td>4</td>
<td>4:6</td>
<td>17.65</td>
<td>&lt;120</td>
</tr>
<tr>
<td>5</td>
<td>5:5</td>
<td>14.40</td>
<td>&lt;90</td>
</tr>
</tbody>
</table>

Fig. 2. Pseudo-ternary Phase Diagram of SMEDDS
Filled area denoted the efficient self-emulsifying domain with the effect of different oils. (a) and (b) represented Crodamol GTCC and Maisine 35-1, respectively; (c), (d) and (e) represented 2:1, 1:1 and 1:2 of Crodamol GTCC, Maisine 35-1 (w/w), respectively. They were all kept with Cremophor RH 40 and 1,2-propanediol at a ratio of 3:2.
ogy of liquid PF-SMEDDS and solid PF-SMEDDS dropping pills after hydration was characterized by negative stain TEM. Microemulsion droplets were spherical (Fig. 4).

Drug Release in Vitro Release kinetics of PF-SMEDDS dropping pills in the media of pH 1.2, 4.0, 6.8 and distilled water were shown in Fig. 5. The results indicated that the pH of media had no effect on the release of puerarin. Therefore, the distilled water was selected for release comparison study.

Drug release profiles of puerarin from PF-SMEDDS dropping pills and Yufengningxin dropping pills were shown in Fig. 6. The results revealed that the cumulative puerarin release of Yufengningxin dropping pills was lower than that of PF-SMEDDS dropping pills in the same aqueous media. The percentage of puerarin release from PF-SMEDDS dropping pills (105.10%) at 10 min was dramatically greater than that of Yufengningxin dropping pills (85.03%) in distilled water. Small particles were able to form from Yufengningxin dropping pills and PF-SMEDDS dropping pills during the release test process. But for PF-SMEDDS dropping pills, PF-SMEDDS dropping pills were easily dispersed to form a microemulsion with smaller particle size droplet under the condition of gentle agitation in dissolve platform, because the free energy required to form an emulsion was very low. The spontaneous formation of microemulsion enabled the drug to be in a dissolved form. The resultant smaller droplet size presented larger interfacial area and advantageously accelerated the drug release rate.

Cremophor RH 40, surfactant in PF-SMEDDS dropping pills could enhance release rate because of
improving drug solubility.

**Bioavailability Study** The mean plasma concentration-time profiles of were presented in Fig. 7 and the pharmacokinetic parameters were listed in Table 4 after oral administration PF-SMEDDS dropping pills or Yufengningxin dropping pills to rats. The value of $t_{1/2}$, $C_{\text{max}}$ and $AUC_{0-6\text{h}}$ showed dramatic difference between PF-SMEDDS dropping pills and commercial Yufengningxin dropping pills. The oral relative bioavailability of puerarin from PF-SMEDDS dropping pills was significantly enhanced 2.36-fold compared with that of Yufengningxin dropping pills (3.322±0.366 mg/L·h vs. 1.429±0.168 mg/L·h, $p<0.05$). The results demonstrated that the absorption of puerarin from PF-SMEDDS dropping pill was enhanced compared to Yufengningxin dropping pills. The maximum plasma concentration ($C_{\text{max}}$) was also improved by 1.69-fold in the PF-SMEDDS dropping pills compared with that of Yufengningxin dropping pills (1.151±0.091 µg/mL·h vs. 0.681±0.05 µg/mL·h, $p<0.05$). The time to reach $C_{\text{max}}$ ($T_{\text{max}}$) of PF-SMEDDS dropping pills resulted in a 1.28-fold increase compared with that of Yufengningxin dropping pills (0.642±0.069 h vs. 0.502±0.030 h, $p<0.05$). The values of $t_{1/2}$ of PF-SMEDDS dropping pills decreased by 1.57-fold.
and $AUC_{0-\infty}$ increased by 1.90-fold compared with that of Yufengningxin dropping pills. From this study, we deduced that several mechanisms might improve the bioavailability of PF-SMEDDS dropping pills.

For PF-SMEDDS dropping pills, sufficient dissolution was a critical factor for absorption in the gastrointestinal tract. PF-SMEDDS dropping pills were able to form a microemulsion with small particle size droplet under the condition of gentle agitation in the dissolving platform. Small particle size droplet under the condition of gentle agitation in the dissolving platform. Small particle size droplet formed a microemulsion critical factor for absorption in the gastrointestinal tract. PF-SMEDDS dropping pills were able to form a microemulsion with small particle size droplet upon gentle agitation in the dissolving platform. Small particle size droplet formed a microemulsion critical factor for absorption in the gastrointestinal tract.

High contents of surfactants (Cremophor RH 40) were inserted into the cell membrane where they could form polar defects in the lipid bilayer and disrupt the structural organization of the lipid bilayer, resulting in a permeation enhancement of the drug. They also decreased the interfacial surface tension and increased puerarin permeability of the intestinal epithelial cells which allowed them to be readily absorbed in the gastrointestinal tract.

CONCLUSION

PF-SMEDDS dropping pills were able to form a microemulsion with uniform fine particle size droplet upon gentle stir in water medium. The dilution volume showed no effect on droplet size or self-microemulsifying behavior. The cumulative in vitro release percent of puerarin from PF-SMEDDS dropping pills was higher than that of Yufengningxin dropping pills. The bioavailability of PF was greatly enhanced by PF-SMEDDS dropping pills. These results demonstrated the potential use of PF-SMEDDS dropping pills as an efficient way to increase the oral absorption of poorly water-soluble drugs.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

Table 4. Pharmacokinetic Parameters of PF-SMEDDS Dropping Pills and Yufengningxin Dropping Pills (Mean±S.D., n=6)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Yufengningxin dropping pills</th>
<th>PF-SMEDDS dropping pills</th>
<th>$t$</th>
<th>$p$ (Sig)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>4.380±1.483</td>
<td>2.791±0.557*</td>
<td>2.456</td>
<td>0.034</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>0.502±0.030</td>
<td>0.642±0.069*</td>
<td>−5.00</td>
<td>0.004</td>
</tr>
<tr>
<td>$C_{max}$ (µg/mL)</td>
<td>0.681±0.053</td>
<td>1.151±0.091*</td>
<td>−10.874</td>
<td>0.000</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (mg/L·h)</td>
<td>1.429±0.168</td>
<td>3.322±0.366*</td>
<td>−11.536</td>
<td>0.000</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (mg/L·h)</td>
<td>2.251±0.588</td>
<td>4.277±0.776*</td>
<td>−5.097</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Significantly different from Yufengningxin dropping pills ($p<0.05$) by Student’s $t$-test.

REFERENCES


