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Characterization and evaluation of self-microemulsifying sustained-release pellet formulation of puerarin for oral delivery

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ABSTRACT

The present study aims to develop self-microemulsifying drug delivery systems (SMEDDS) in sustained-release pellets of puerarin to enhance the oral bioavailability of puerarin. The performances of puerarin–SMEDDS including oils, emulsifiers, and co-emulsifiers were evaluated. Pseudo-ternary phase diagrams shows that the optimized formulation consisted of castor oil as the oil phase, Cremophor® EL as the emulsifier, and 1,2-propanediol as the co-emulsifier. SMEDDS sustained-release pellets were prepared via extrusion–spheronization. The particle size distributions of the formulations were determined using transmission electron microscopy and scanning electronic microscopy. The mean particle size was 50 ± 8 nm. The pharmacokinetics and bioavailability of the puerarin–SMEDDS sustained-release pellets and puerarin tablets were evaluated and compared in beagle dogs. The absolute bioavailability of the puerarin–SMEDDS sustained-release pellets was enhanced by approximately 2.6-fold compared with that of the puerarin tablet. The relative bioavailability (F_{rel}) of the SMEDDS pellets was 259.7% compared with the tablet group. The results demonstrated that the puerarin–SMEDDS sustained-release pellets had a sustained-release effect, and could remarkably improve the oral bioavailability of puerarin.

1. Introduction

Puerarin or 4,7-dihydroxy-8β-D-glucosyl isoflavone is practically water insoluble and is abundant in the traditional Chinese medicine kudzuvine root *Pueraria lobata* (Luo et al., 2011; Chen and Chan, 2009; Wang and Song, 2004). Puerarin has many beneficial properties related to cardiovascular conditions, such as hypertension, hyperlipidemia, hemicrania, coronary heart disease, myocardial infarction, and angina pectoris (Barthe et al., 1998; Shen et al., 2007). Puerarin can also improve microcirculation, expand the coronary artery, and increase the blood flow in the brain as well as coronary artery. Puerarin also has anti-thromboxane, antispasm and anti-platelet aggregation properties as well (Zhao and Xiang, 2000; Yeung et al., 2006; Chung et al., 2008; Hsu et al., 2003). Puerarin is currently available in the market as oral preparations, such as pellets, granules, and capsules. However, these preparations have low bioavailability (Wu et al., 2011), which results in the high daily doses and poor compliance. Hence, a novel oral preparation of puerarin needs to be developed.

Self-microemulsifying drug delivery systems (SMEDDS) are mixtures of oils, surfactants, and co-surfactants (Schamp et al., 2006; Kovarik et al., 1994). Several studies have shown that lipophilic compounds are better absorbed when administrated in self-microemulsifying formulations (Chae et al., 2005; Sousa et al., 2002). SMEDDS can significantly improve the bioavailability of insoluble and hydrophobic drugs. SMEDDS possess higher solubilization, more stable physicochemical properties, and easier preparation process than other normal formulations (Constantinides and Yiv, 1995). Pellets offer several advantages of oral sustained-release drug delivery systems. Because they travel within the gastrointestinal tract (GIT) under less variable transit times than other forms (Fukumori, 1997). Over the past few years, studies on either self-microemulsifying pellets (Setthacheewakul et al., 2010) or controlled release pellets (Serratoni et al., 2007) have been extensive. However, studies on SMEDDS sustained-release pellets are limited.

The present study aims to (i) prepare and characterize a SMEDDS sustained-release pellet formulation for oral puerarin delivery, (ii) study the release and formulation stability of puerarin–SMEDDS sustained-release pellets in vitro, and (iii) determine whether the SMEDDS sustained-release pellet formulation can improve the oral bioavailability of the poorly water-soluble drug puerarin. Puerarin–SMEDDS sustained-release pellets were prepared via a self-microemulsifying and extrusion–spheronization method (Fu et al., 2011). The physicochemical characteristics of the puerarin–SMEDDS sustained-release pellets were assessed by transmission electron microscopy (TEM), scanning electronic microscopy (SEM), and photon correlation spectroscopy. The

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release of the puerarin–SMEDDS sustained-release pellets in vitro was studied using a dissolution apparatus. The relative bioavailability of the puerarin–SMEDDS sustained-release pellets was also studied in beagle dogs.

2. Materials and methods

2.1. Materials

Cremophor® EL (CrEL) was obtained from BASF (Ludwigshafen, Germany). Puerarin (98% pure) was purchased from China National Drug Control (Beijing, China). Castor oil, isopropyl myristate (IPM), labrafacv (Lab), soybean oil, oleic acid, ethanol, 1,2-propanediol, polyethylene glycol 400 (PEG 400), Tween 80, and Span 80 were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Microcrystalline cellulose (MCC) was obtained from the Shanghai Chinaway Pharmaceutical Tech. Co., Ltd. (Shanghai, China). Hydroxypropyl methyl cellulose (HPMC) was purchased from the Dalian Diligence Trade Co., Ltd. (Dalian, China). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Solubility studies

The solubility of puerarin in different media, such as oils (castor oil, Lab, soybean oil, oleinic acid, and IPM), emulsifiers (Tween 80, CrEL, and Span 80), and co-emulsifiers (propylene glycol, alcohol, PEG 400, and glycerin) was evaluated. An excess amount of puerarin was added to 5 ml of the dissolution medium, which comprises an oil phase, emulsifier, and co-emulsifier. The samples were incubated in an air-bath oscillator at 37 ± 0.5 °C for 72 h until equilibrium. The mixtures were then centrifuged at 15,000 rpm for 10 min. The supernatant was removed and diluted by methanol, and then analyzed for puerarin content using HPLC (Section 2.2.9).

2.2.2. Construction of pseudo-ternary phase diagrams

The emulsifier and co-emulsifier were weighed precisely and mixed at mass ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. For each pseudo ternary phase diagram at a specific emulsifier/co-emulsifier weight ratio, the oily mixtures of oil, emulsifier and co-emulsifier were prepared with the weight ratio of oil to the mixture of emulsifier and co-emulsifier at 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9, respectively. The mixture was homogeneously mixed using a vortex mixer (QL-901, Kylin-Bell Lab Instruments Co., Ltd., China). Water was added to the mixtures, and the samples were stirred for homogenization. All the samples were then monitored against a dark background. The microemulsion regions in the phase diagrams were identified, and the microemulsion formulations were selected at desired component ratios.

2.2.3. Preparation of puerarin–SMEDDS

Variable proportions of oil, emulsifiers and co-emulsifiers were added into a 10 ml screwcapped glass tube, and the components were mixed by gentle stirring. Puerarin was then dissolved in the resulting mixture by stirring magnetically at room temperature. The contents of oil, emulsifiers and co-emulsifiers were chosen at the range of 20%, 40–60% and 20–40%, respectively, in order to obtain the optimal formulation of SMEDDS.

2.2.4. Formulation and preparation of puerarin–SMEDDS sustained-release pellets

Based on the results of pseudo ternary phase diagrams and the solubility, the optimal formulation of SMEDDS was obtained, according to the reported method (Wang et al., 2010; Siddique et al., 2010; Serratoni et al., 2007), the formulation was selected and puerarin–SMEDDS sustained-release pellets were prepared through extrusion–spheronization. The formulation contained 3% puerarin, 5.4% castor oil, 13.5% CrEL, 8.1% 1,2-propanediol, 60% HPMC, and 10% MCC. The formulation was prepared by first adding 200 ml of 75% ethanol to a mixture containing 3 g of puerarin, 5.4 g of castor oil, 13.5 g of CrEL, and 8.1 g of 1.2-propanediol. Mixing was performed until a homogeneous solution was obtained. Approximately 60 g of HPMC was well-mixed with 10 g of MCC. This mixture was added to the puerarin mixture, and a soft material was obtained. This material was pelletized using an extrusion–spheronization machine (Guanlian Pharmaceutical Equipment Co., Ltd., China). The wet mass was passed through an extruder at 30 rpm. The extrudates were then placed in a spheronizer fitted with a crosshatched plate rotated at 1800 rpm for 10 min.

2.2.5. Physical characterization of puerarin–SMEDDS sustained-release pellets

The morphology of the optimum self-microemulsifying pellets was observed by TEM (JEOL-2010, JEOL, Tokyo, Japan). About 0.1 g of the puerarin of self-microemulsifying solution and 0.3 g of the puerarin self-microemulsifying pellets were separately placed in beakers. About 30 ml of distilled water was added to the beakers, and after sonication (100 W for 3 min), the solution was filtered through a microporous membrane (0.22 μm). The samples were dropped on copper grids for 1–2 min, the excess was removed by filter paper, and then the copper grids were placed in 1% phosphotungstic acid for about 30 s. The excess phosphotungstic acid was then removed. TEM micrographs of the puerarin microemulsions were obtained.

The morphology of the pellets, including their external surfaces and the cross-sections was investigated by SEM (FEI Sirrion 200/InCa Oxford, Oxford, UK). The pellet formulations were mounted on the stub. The specimen was then sputter-coated with gold particles. The integrated and cross-cutting pellets were adhered to the fixer, and placed into the scanning electron microscopy for SEM analysis.

The particle sizes of the puerarin–SMEDDS sustained-release pellets were measured through photo correlation spectroscopy using a particle size analyzer (BIC 90 Plus, Brookhaven Instruments Corporation, USA) at a fixed angle of 90° at 25 °C.

2.2.6. Release of puerarin–SMEDDS sustained-release pellets

The release profiles of the pellets filled with the puerarin–SMEDDSs, the SMEDDSs of puerarin, and the puerarin powder (all these formulations contained 10 mg of puerarin) were studied using a dissolution apparatus (ZRS-8G, Tianjin University Electronics Co., Ltd., China).

The formulations of puerarin–SMEDDS sustained-release pellets with different ratios of the excipients, the puerarin–SMEDDS, and the puerarin powder were separately placed in 900 ml of phosphate buffer (pH 6.8). In vitro release experiments were performed by the rotary basket method at a rate 50 rpm and 37 ± 0.5 °C. The release profiles of the three formulations of puerarin–SMEDDS sustained-release pellets were compared to the release profiles of the unformulated puerarin and the puerarin–SMEDDS.

Samples (0.5 ml) were drawn and replaced with new fresh media at the predetermined points. The samples were then filtered and analyzed using the HPLC assay as described in Section 2.2.9. Three separate replicate experiments were conducted for each formulation, the data presented are the means ± SD (n = 3).

2.2.7. Stability studies

Stable SMEDDS sustained-release pellets can be generally stored for a long time under normal storage conditions. Properties such as content and, pellet size after dilution should not be significantly

changed. In the current research, the stability of the sustained-release pellets was evaluated for three months.

The samples of puerarin self-microemulsifying sustained-release pellets were maintained in a stability chamber at 30 ± 2 °C and 65 ± 5% relative humidity (RH). The samples were collected after 0, 1, 2 and 3 months. The appearance, self-emulsifying properties, emulsion size, and drug content were evaluated.

2.2.8. Bioavailability

Six healthy beagle dogs (12 ± 2 kg), fasted but free access to water for 12 h prior to the experiment, were used in the study. They were administered orally prepared test puerarin–SMEDDS pellets or formulation tablets (5 mg/kg) with 100 ml water. The washout period between the consecutive treatment schedules was 1 week. To compare oral bioavailability with intravenous injection, 2.5 mg/kg puerarin suspension was intravenously injected into the forward limb vein (Cui et al., 2005; Yu et al., 2010). The animal experimental protocols were performed according to the guidelines of the Experimental Animal Ethics Committee of Shanghai Jiao Tong University. The beagle dogs were randomly divided into three groups, and separately treated with the different dosage forms.

After administration, 1 ml of blood samples were collected from forelimb vein of beagle dogs at different predetermined time points of 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 540, and 600 min. The samples were then placed in heparinized microcentrifuge tubes (100 IU/ml blood) and centrifuged at 5000 rpm for 5 min. The separated plasma samples were stored at −80 °C until analysis.

Puerarin concentration in the plasma with 0.05 μg/ml, 4-hydroxybenzaldehyde as the internal standard (IS) was determined by the HPLC method as mentioned in Section 2.2.9. The plasma samples were prepared by first mixing 100 μl of plasma with 50 μl of the internal standard. About 900 μl of acetonitrile was added to the plasma, and the mixture was vortexed for 5 min. The plasma sample was centrifuged at 12,000 rpm for 10 min. The supernatant was collected and dried by nitrogen gas. The residue was then redispersed in 100 μl of methanol. Following further centrifugation at 12,000 rpm for 10 min, 20 μl of the sample solution was injected into the HPLC system. The peak concentration (C_{max}) and the time to reach the peak concentration (T_{max}) were directly determined from the plasma concentration–time curves. The area under the curve (AUC) was calculated by the trapezoidal method from zero to the final sampling time.

Bioavailability was studied by comparing two different formulations, namely, the SMEDDS pellets and tablets of puerarin. The relative bioavailability of the SEMDDS pellets of puerarin was calculated by the following equation:

$$F_{rel} = \frac{AUC_{test}/Dose_{test}}{AUC_{reference}/Dose_{reference}} \times 100\%$$

where F_{rel} is the relative bioavailability, and AUC_{test} and $AUC_{reference}$ are the AUCs of the test and reference formulations, respectively.

2.2.9. HPLC analysis

The quantitative determination of puerarin was performed using an HPLC equipment (Shimadzu, Japan) consisting of a LC-20AT HPLC pump and an SPD-20A UV-VIS detector. The analysis of puerarin was conducted on a Dikma Diamonsil C_{18} column (150 mm × 4.6 mm i.d. 5 μm, Dikma Technologies, China). The mobile phase was composed of methanol and 0.02 M NaH_2PO_3 at a volume ratio of 25:75 (Chen et al., 1999). The mobile phase was pumped at a flow rate of 1.0 ml/min. The detection wavelength was set at 250 nm and the injection volume was 20 μl.

3. Results and discussion

3.1. Solubility studies

Solubility and permeability are the fundamental determinants of the oral bioavailability of a drug (Varma et al., 2004). The results of puerarin solubility in various media are listed in Table 1. Solubility in castor oil was 127.82 ± 22.17 μg/ml, which is much higher than those in other oil phases. Solubility in 1,2-propanediol was 114.37 ± 26.56 mg/ml, which was the highest among all co-emulsifiers. Solubility in Tween 80 and CrEL were 40.46 ± 8.1 and 29.50 ± 3.97 mg/ml, respectively. The puerarin–SMEDDS consisting of oil, emulsifier, and co-emulsifier, in which the solubility of puerarin was higher than the others, may enhance the bioavailability of puerarin by improving its solubility and permeability.

3.2. Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed to identify the microemulsion regions and optimize the vehicles (oil, emulsifier, and co-emulsifier). The best ratios of the excipients obtained from these diagrams were used to obtain the microemulsion regions area (Section 2.2). In a traditional oil/water SMEDDS, a non-ionic emulsifier with a high hydrophilic–lipophilic balance is likely to be used for its drug compatibility, strong self-microemulsion ability, low toxicity, and hemolysis (Spernath and Aserin, 2006). Fig. 1 shows the phase diagrams of systems with different oil phases (castor oil and Lab), because the solubility of puerarin in castor oil and lab are much higher than other oils, so castor oil and lab was selected as oil phase; emulsifiers (Tween 80 and CrEL); and co-emulsifiers (1,2-propanediol and PEG 400), due to the volatile nature, ethanol was eliminated as a co-surfactant.

As shown in Fig. 1, when CrEL was used as the emulsifier and 1,2-propanediol as the co-emulsifier, the microemulsion areas were similar with those when either chosen castor oil or Lab was chosen. Castor oil was used as the oil phase for SMEDDSs because of its high miscibility. When castor oil was used as the oil phase and 1,2-propanediol was the co-emulsifier, the microemulsion area with CrEL as the emulsifier was much larger than that with Tween 80, Cremophor EL was a good emulsifier for the drug used in microemulsion, it maybe provide reduction in the surface tension and fluidizes the interfacial surfactant film which can expand the area of existence of microemulsion system (El Maghraby, 2008). When a certain proportion of the oil phase and emulsifier (castor oil and CrEL) was fixed, the microemulsion area using PEG 400 was a little larger than that when 1,2-propanediol was used but not to a significant extent. 1,2-Propanediol can extremely reduce the surface tension and viscosity of SMEDDSs, thereby, effectively preventing SMEDDSs from turning into gel (Gelderblom et al., 2001). 1,2-Propanediol was used as the co-emulsifier of SMEDDS based on the foregoing and the results of solubility test results. As shown in the diagrams, the effect of castor oil and Lab on the microemulsion area was not significant. Using CrEL as the emulsifier remarkably affected on the area of microemulsion. Therefore, the system of castor oil – CrEL – 1,2-propanediol was selected for the development of SMEDDS formulations.

3.3. Formulation and preparation of puerarin–SMEDDS

The pseudo-ternary phase diagrams showed that the oils, emulsifiers, and co-emulsifiers, which can form a large area of self-microemulsion, were mixed at ratios of 2:6:2, 2:5:3, and 2:4:4, respectively. The microemulsion size and solubility of puerarin were examined.

The oils slightly affected the microemulsion size. However, the size decreased when 1,2-propanediol was used as the emulsifier.

Table 1
Solubility of puerarin in various oils, emulsifiers, and co-emulsifiers. Data represent the means ± SD ($n = 3$).

Oils	Solubility (μg/ml)	Co-emulsifiers	Solubility (mg/ml)	Emulsifiers	Solubility (mg/ml)
Castor oil	127.82 ± 22.17	1,2-Propanediol	114.37 ± 26.56	Tween 80	40.46 ± 8.14
Lab	14.29 ± 3.12	Alcohol	75.68 ± 14.03	CrEL	29.50 ± 3.97
Soybean oil	2.14 ± 0.27	PEG 400	49.75 ± 7.99	Span 80	1.94 ± 0.21
Oleinic acid	1.58 ± 0.13	Glycerin	46.58 ± 8.49		
IPM	0.58 ± 0.13				

Puerarin solubility was higher when CrEL was combined with 1,2-propanediol than with other co-emulsifiers. The highest solubility was obtained when the ratio of CrEL to 1,2-propanediol was 5:3. The optimal ratio of the oil phase, emulsifier, and co-emulsifier was set to 2:5:3 after comparison with other formulations, which considered both the high solubility and large area of the self-microemulsion.

Other factors also affected the efficiency of the self-microemulsification. In the present study, the self-microemulsification time was much shorter when phosphate buffer was used as the dilution medium than when other normal media were used. The self-microemulsification time was also clearly shortened when the magnetic stirring speed was increased (data not shown).

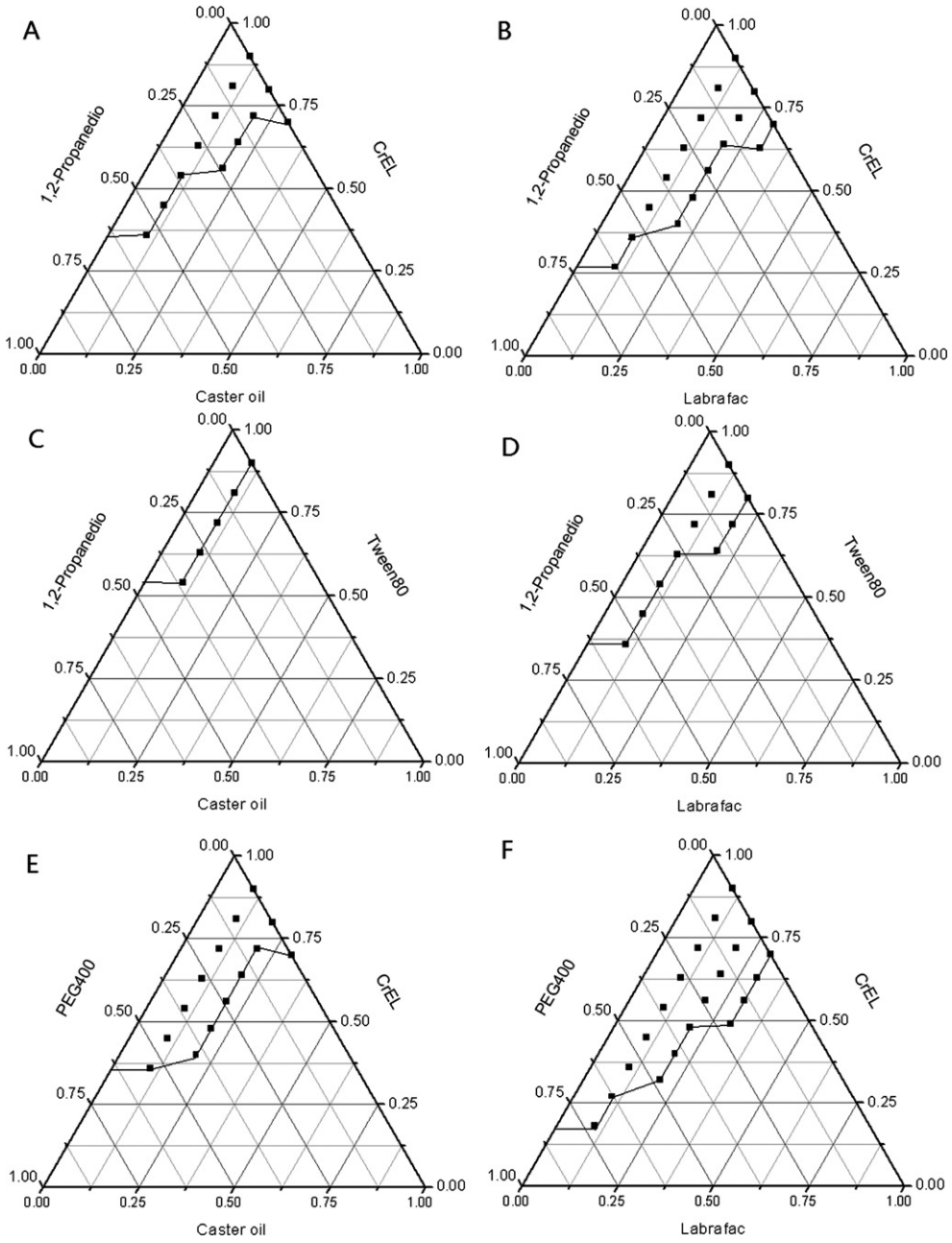

Fig. 1. Pseudo-ternary phase diagrams composed of various oils, emulsifiers and co-emulsifiers. The formulations of SMEDDSs were as follow: castor oil–CrEL–1,2–propanediol (A), Lab–CrEL–1,2-propanediol (B), castor oil–Tween 80–1,2–propanediol (C), Lab–Tween 80–1,2-propanediol (D), castor oil–CrEL–PEG 400 (E), and Lab–CrEL–PEG 400 (F).

Table 2
Compositions of different puerarin–SMEDDS sustained-release pellet formulations.

Ingredients	Formulations (% w/w)		
	1	2	3
Puerarin	3	3	3
Castor oil	5.4	5.4	5.4
CrEL	13.5	13.5	13.5
1,2-Propanediol	8.1	8.1	8.1
HPMC (15,000 sr)	60	50	40
MCC	10	20	30

3.4. Preparation and release of puerarin–SMEDDS sustained-release pellets

The selected optimum preparation conditions were as follows: speed of extrusion was 30 rpm, speed of spheronization was 1800 rpm and time of spheronization was 10 min. Three formulations of puerarin–SMEDDS sustained-release pellets (Table 2) were prepared. These formulations contained a fixed proportion of puerarin–SMEDDS and different ratios of excipients (HPMC and MCC). Puerarin–SMEDDS, puerarin–SMEDDS sustained-release pellets with various ratios of HPMC and MCC, and unformulated puerarin were used for the release experiments. The release profiles are shown in Fig. 2. Puerarin was rapidly released from puerarin–SMEDDS, and an almost complete release was achieved within 1 h. On the other hand, all cumulative released rates of puerarin–SMEDDS sustained-release pellets were above 90% within 600 min, compared with a release rate of less than 30% release from the unformulated puerarin within 600 min. The release process was slow and uniform. Whether these formulations could lead to initial burst release was not proven. The three release profiles of puerarin–SMEDDS sustained-release pellets were slightly different some parts, but the general trends were similar to each other (Fig. 2). Increasing the proportion of HPMC may lead to a low release speed. Therefore, the best sustained-release effect can be achieved when the SMEDDS:HPMC:MCC ratio is 3:6:1. The puerarin–SMEDDS pellets had a better sustained-release effect than the others. Using 75% ethanol as the adhesive results in a much better performance and a good sustained-release effect of HPMC (a kind of hydrophilic gel) under higher water content (Wang et al., 2010). Consequently, the behavior of puerarin release from puerarin–SMEDDS sustained-release pellets in the present study indicates that SMEDDS pellets may have significantly increased puerarin dissolution compared with other formulations. An extension of the release time of up to 600 min has also been effectively conducted. Therefore, the formulation with 3:6:1 SMEDDS–HPMC–MCC was selected to obtain a sustained-release effect and achieve a reduction in the drug release time.

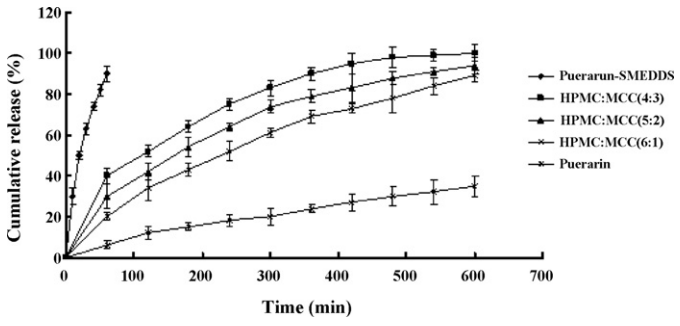

Fig. 2. Cumulative released percent of puerarin from puerarin–SMEDDS sustained-release pellets with different ratios of HPMC and MCC, puerarin–SMEDDS, and puerarin. Data are presented as means ± SD ($n = 3$).

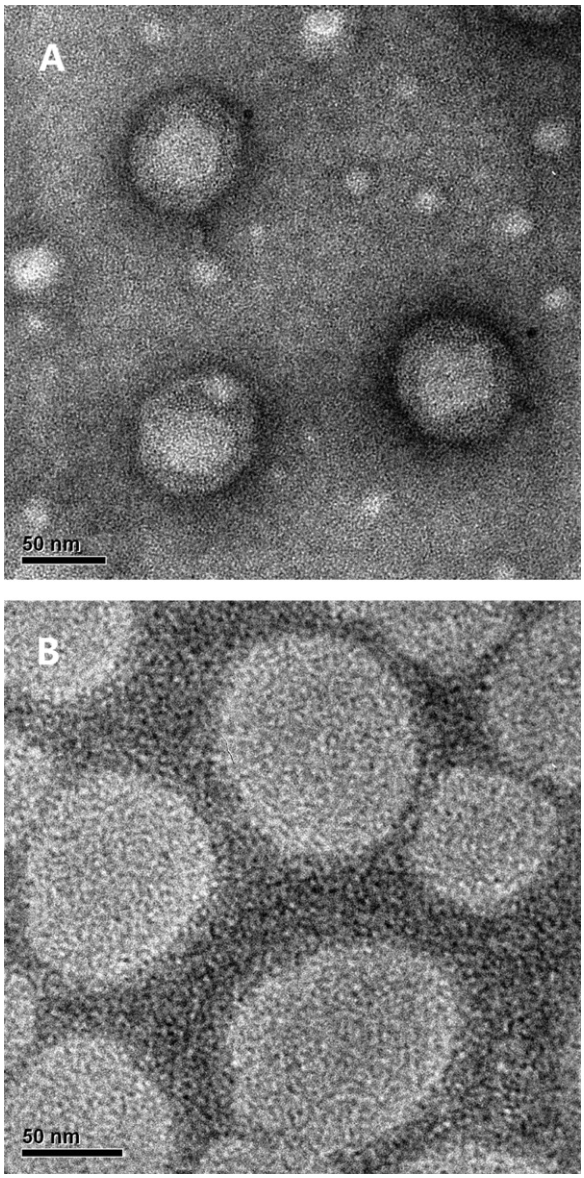

Fig. 3. TEM images of puerarin–SMEDDS (A) and SMEDDS sustained-release pellets (B).

3.5. Physical characterization of puerarin–SMEDDS sustained-release pellets

The results of TEM and the mean diameters of the sustained-release pellets are shown in Fig. 3. Both mean sizes were about 50 ± 8 nm, and no obvious difference was found on the particle size between puerarin–SMEDDS and SMEDDS sustained-release pellet. This result reveals that sustained-release pellets can form microemulsions and that the microemulsion size is not affected by excipients.

The pellet surface and cross-sections were studied by SEM. Fig. 4 shows that the pellets had a spherical shape and smooth surface. The mean diameter of the pellets was 500 μm. The formation process of microemulsion was affected by HPMC, MCC, and the preparation method. The pellet size increased with increasing spheronization speed and time. MCC, which possesses capillarity, expanded as a "molecular sponge" when water was introduced into the system. Consequently, appropriate elasticity and plasticity were achieved. This result proves that MCC is a good spherulization accelerating agent (Tang et al., 2008).

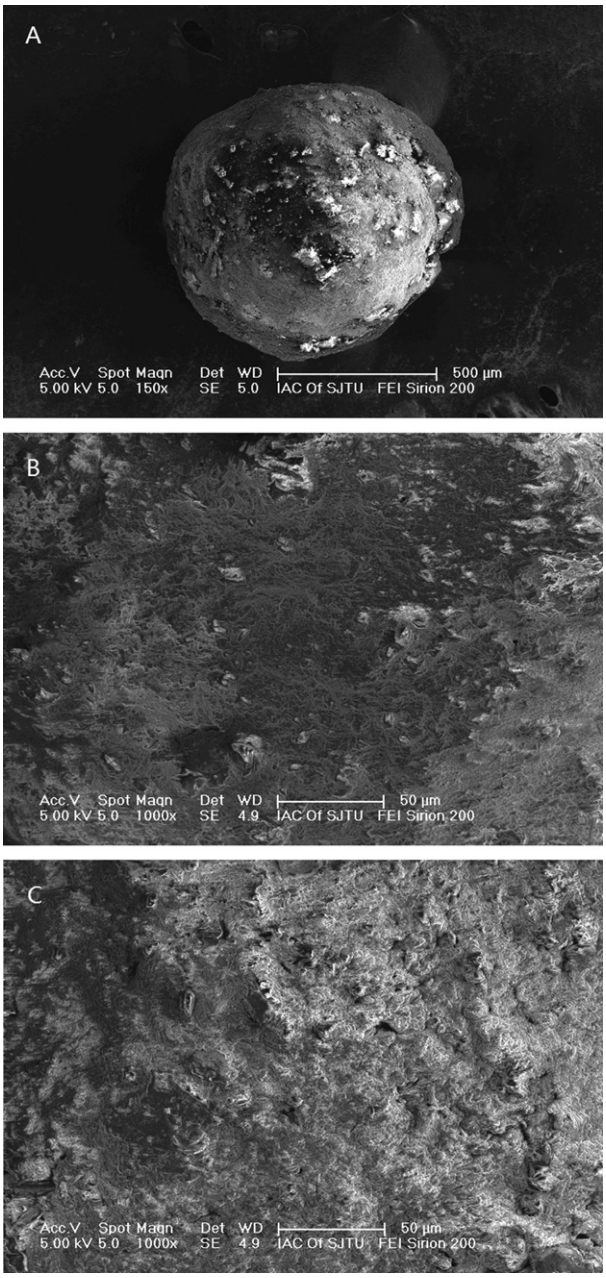

Fig. 4. SEM micrographs of surfaces and cross-sections of puerarin–SMEDDS sustained-release pellets. A (150×) and B (1000×) surface of pellets, C (1000×) cross-section of pellets.

3.6. Drug content

Puerarin characterization in the pellets was conducted as follow: about 0.02 g of puerarin–SMEDDS sustained-release pellets were precisely weighed and placed in a 100 ml volumetric flask.

Table 3
Stability data of puerarin–SMEDDS sustained-release pellets. Data reported are means ± SD ($n = 3$).

Time (month)	Appearance	Mean size (nm)	Content (%)
0	Yellow spherical pellets	48.0 ± 5.0	99.90 ± 2.34
1	Yellow spherical pellets	47.0 ± 3.0	99.23 ± 1.87
2	Yellow spherical pellets	47.5 ± 2.0	99.49 ± 2.06
3	Yellow spherical pellets	48.4 ± 4.0	99.38 ± 1.98

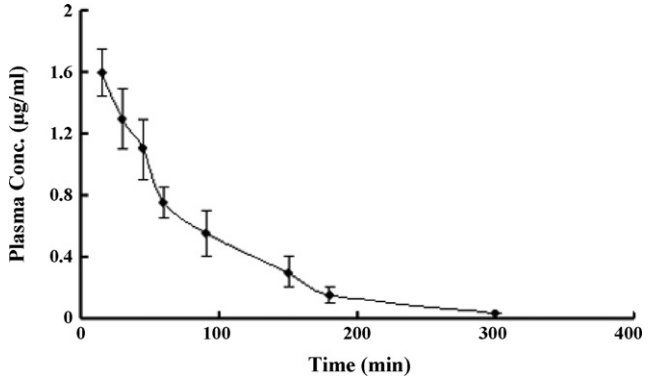

Fig. 5. Plasma concentration–time profile of puerarin after intravenous administration in beagle dogs. Results are expressed as the means ± SD ($n = 6$).

The pellets were then diluted with the mobile phase. The appropriate solution was collected and centrifuged at 10,000 rpm for 10 min. The supernatants were then removed and detected by HPLC (Section 2.2.9). The theoretical content of puerarin in the sustained-release pellets was 3% whereas the actual content was 2.997%. The ratio of the actual to theoretical content was 99.9%.

3.7. Stability studies

The puerarin–SMEDDS sustained-release pellets were kept at 30 ± 2 °C and 65 ± 5% RH, for three months. The results are shown in Table 3. The puerarin content in the SMEDDS remained at about 99%, which reflected that the optimized formulation was stable under the experiment condition. Furthermore, no significant change was found in the appearance, puerarin content, and particle size.

3.8. Bioavailability

The SMEDDS pellet or tablet was orally administrated to beagle dogs. Puerarin was intravenously injected into the forward limb vein.

Puerarin was rapidly distributed after intravenous administration (Fig. 5). The T_{max} was 20 min, which is shorter than that of puerarin–SMEDDS sustained-release pellet. The plasma concentration–time curves profiles of the puerarin tablet and puerarin–SMEDDS sustained-release pellets are shown in Fig. 6.

Table 4 shows the pharmacokinetic parameters [C_{max}, T_{max}, AUC, mean resident time (MRT), and bioavailability] of puerarin after oral administration. C_{max} was higher for the SMEDDS pellet (0.33 ± 0.02 μg/ml) than that for the tablet (0.27 ± 0.05 μg/ml). Similarly, MRT was larger for the SMEDDS pellet (185 ± 26.31 min)

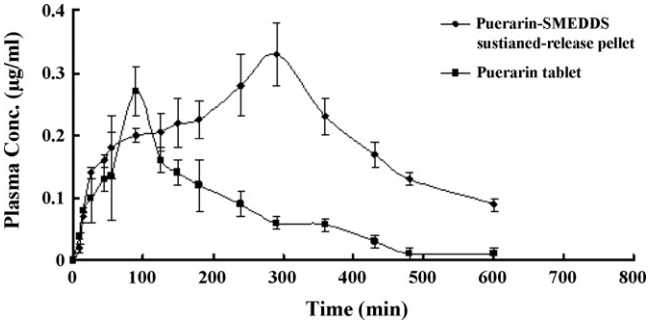

Fig. 6. Plasma concentration–time profiles of puerarin–SMEDDS sustained-release pellets and puerarin tablet after oral administration in beagle dogs. Results are expressed as the means ± SD ($n = 6$).

Table 4
Pharmacokinetic parameters of puerarin after intravenous (2.5 mg/kg) or oral (5 mg/kg) administration in beagle dogs ($n = 6$).

Pharmacokinetic parameters	Injection	Tablet	Pellet	Ratio
$AUC_{0\to t}$ (μg h/ml)	220.87 ± 13.40	57.78 ± 23.27	150.06 ± 27.92[b]	2.60
MRT (min)	51 ± 7.8	74 ± 11.63	185 ± 26.3[b]	2.50
C_{max} (μg/ml)	1.48 ± 0.07	0.27 ± 0.05	0.33 ± 0.02	1.30
T_{max} (min)	20 ± 2.3	90 ± 7.5	280 ± 30[b]	
Cl (ml/min)	108.17 ± 15.49	373.46 ± 79.51	134.35 ± 24.50[a]	
Absolute bioavaibility (%)	–	13.08	33.97	
Relative bioavaibility (%)	–	–	–	259.70

[a] $p < 0.05$ compared with the tablet.
[b] $p < 0.01$ compared with the tablet.

than for the tablet group (74 ± 11.63 min). T_{max} is generally believed to be responsible in releasing the drug from a microemulsion. T_{max} is determined from experimental measurements because the mechanism of absorption enhancement is still unclear. Table 4 shows that T_{max} was also higher for the SMEDDS pellet (280 ± 30 min) than for the tablet group (90 ± 7.5 min). Similarly, the $AUC_{0\to t}$ of the SMEDDS pellet (150.06 ± 27.92 μg h/ml) was 2.6-fold higher than that of the tablet (57.78 ± 23.27 μg h/ml). Finally, the absolute bioavailabilities of the SMEDDS pellet and tablet of puerarin were 33.97% and 13.08%, compared with intravenous injection group, respectively. The relative bioavailability (F_{rel}) of the SMEDDS pellet was 259.7% compared with the tablet. These results verify that the SMEDDS pellet is effective in improving the oral bioavailability of puerarin.

The plasma concentration of the puerarin tablet clearly reached C_{max} within 90 min after oral administration, and then rapidly decreased. Compared with the plasma concentration profile of the puerarin tablet, the plasma concentration profile of the puerarin–SMEDDS sustained-release pellet was a little faster during the first 60 min, and gradually increased. The peak was reached after 280 min. The self-microemulsification ability of puerarin–SMEDDS sustained-release pellets that occurs within the first 60 min is suggested to result in a relatively faster drug release. The slowly increasing curve may be attributed to the sustained release effect in puerarin–SMEDDS sustained-release pellets.

Drug release in SMEDDS pellets was much slower than that of the tablets. The curves also clearly illustrated a significant difference between the bioavailability in the SMEDDS pellet and tablet.

In the present case, a remarkable increase in the AUC of puerarin was observed when the SMEDDS pellet was studied. The results revealed that the formulation of puerarin as an SMEDDS sustained-release pellet significantly promoted and sustained puerarin absorption. One unique property of SMEDDS is that it has better absorption in the gastrointestinal tract when loaded with lipophilic drugs (Lu et al., 2008). An optimized formulation involving castor oil, CrEL, 1,2-propanediol, MCC, and HPMC, which could enhance the solubility of puerarin, can also improve absorption. The small droplet size (in the range of 50 nm) of the microemulsions may penetrate the absorption site via the transcellular pathway, and could protect the drug from enzyme degradation (Gursoy and Benita, 2004). Therefore, the higher bioavailability in puerarin–SMEDDS sustained-release pellet may be due to the enhanced absorption through the lymphatic pathway, as previously reported (Wu et al., 2006; Gershanik and Benita, 2000). A relatively high ratio of emulsifier in SMEDDS may also contribute to the increased permeability by disturbing the cell membrane (Swenson and Curatolo, 1992).

4. Conclusions

An improved formulation of puerarin–SMEDDS sustained-release pellet was successfully developed in the present study. The in vitro release rate of puerarin from the SMEDDS sustained-release pellets was much slower than that when puerarin was dissolved in liquid SMEDDS. Stability tests showed that the puerarin–SMEDDS sustained-release pellet was stable for three months. The absolute bioavailability of the puerarin–SMEDDS sustained-release pellet showed about 2.6-fold increase in absorption. The relative bioavailability (F_{rel}) of the SMEDDS pellet was 259.7%. Both absolute and relative values were compared with those of the puerarin tablet. Overall, the SMEDDS sustained-release pellet effectively improved the oral bioavailability of puerarin. The formulation had a significant sustained-release effect, and the bioavailability of puerarin dramatically increased.

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