Enhanced Oral Bioavailability of Daidzein by Self-Microemulsifying Drug Delivery System

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To enhance oral absorption of poorly water-soluble daidzein, self-microemulsifying drug delivery system (SMEDDS) composed of oil, surfactant and cosurfactant for oral administration of daidzein was formulated, and its physicochemical properties and pharmacokinetic parameters were evaluated. Solubility of daidzein was determined in various vehicles. Pseudo-ternary phase diagrams were constructed to identify the efficient self-microemulsification region and particle size distributions of the resultant microemulsions were determined using a laser diffraction sizer. From these studies, an optimized formulation consisting of Ethyl oleate (10%), Cremophor RH 40 (60%), and polyethylene glycol 400 (PEG400) (30%) was selected. The dissolution rate of daidzein from SMEDDS was significantly higher than the conventional tablet. Relative bioavailability of SMEDDS was enhanced about 2.5-fold compared with that of the control group. The data suggest that the use of SMEDDS provide a potential way of daidzein administered orally.

Key words self-microemulsifying drug delivery system; daidzein; microemulsion; pseudo-ternary diagram; oral absorption; bioavailability

Oral administration is the preferred route by virtue of its convenience and better compliance. However, the development of this delivery system is considered to be a great challenge due to poorly water-soluble, poorly permeability and rapid metabolism of some drugs. A variety of formulation strategies have been developed to improve the solubility and bioavailability of such drugs. Several approaches such as absorption enhancers,[1,2] chemical modification[3,4] and dosage forms[5,6] have been explored in order to attain peroral delivery of drugs. Lipid-based formulations such as drug incorporation into oils,[7] emulsions[8] and in particular self-microemulsifying formulations[9,10] are known to be successful.

Self-microemulsifying drug delivery system (SMEDDS) are defined as isotropic mixtures of oil, surfactant, co-surfactant and drug that rapidly form a microemulsion upon mixing with water. Self-microemulsifying formulations spread readily in the gastro intestinal (GI) tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification.[11,12] The advantages of these systems include not only improved drug solubility, but also enhanced dissolution and absorption properties, due to the already dissolved form of the drug in the formulation and the resulting small droplets size, providing a large interfacial surface area.[13]

Daidzein (4′,7-dihydroxylisoflavone), a water-insoluble isoflavone, is mainly present in leguminous plants, especially in soybeans, soy foods and Pueraria lobata Ohwi (Leguminosae).[14—16] In recent years, it has been reported that daidzein exhibits a variety of beneficial effects on human health,[17—19] including chemoprevention of cardiovascular diseases such as hypertension, coronary heart disease, atherosclerotic[20] and cancer as well as an alternative for estrogen replacement therapy (ERT) to prevent and treat osteoporosis.

In this study, a SMEDDS formulation of daidzein was developed to improve its oral bioavailability, and discussed the effect of microemulsion particle size and dissolution characteristics on absorption.

Experimental

Materials The daidzein were provided by Qingze Co., Ltd. (Nanjing, China), the purity of these drugs was 98%, which were verified using HPLC. Daidzein tablets (containing 25 mg daidzein in one tablet, batch no. H44020958) were manufactured by Guangzhou Baiyunshan Pharmaceutical Co. (Guangzhou, China). Tween 80, propylene glycol, polyethylene glycol 400 (PEG400), dehydrated alcohol, ethyl oleate, HPLC-grade methanol were purchased from Shanghai Chemical Reagents Institute (Shanghai, People's Republic of China). Cremophor EL and RH40 were purchased from BASF (Germany). Deionized water was prepared by a Milli-Q purication system (Millipore, U.S.A.). All other chemicals were reagent grade.

Solubility Studies Excess daidzein was added to water, acidic water, various oils, surfactants, or cosurfactants and mixed with an ultrasonic cleaner at 40 °C in a water bath for 20 min, then shaken at 25 °C for 48 h. Samples were filtered through a 0.45 μm membrane filter. The filtrate was diluted with methanol and the concentration of daidzein was determined by HPLC.

Pseudo-Ternary Phase Diagram Study Pseudo-ternary phase diagrams were constructed by progressive titration of the component mixtures, both in the absence and presence of the drug. Initially the mixture of Surfactant/CoSurfactant (S/CoS) was used at certain ratio and the oily-phase was added to such mixture in different amounts: 3, 10, 20, 30, 40, 50, 60, 70% (v/v).[21—23] Each mixture was then titrated by adding water up to clouding. The experiments were repeated at different S/CoS (v/v) ratios (1 : 0.5, 1 : 1, 1 : 2, 1 : 3 (w/w)). After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired component ratios.

Preparation of SMEDDS A series of SMEDDS were prepared in each of four formulations (Table 2) with varying ratio of oil, surfactant, cosurfactant, and daidzein. Since daidzein was difficult to dissolve, it was better to dissolve daidzein first by cosurfactant. Then oil and surfactant were added slowly with gentle stirring until homogeneous mixture formed. The mixture was sealed in glass vial and stored under ambient temperature.

Microemulsion Droplet Size Analysis and Morphology Observation by Transmission Electron Microscopy (TEM) Daidzein SMEDDS was diluted with water to a definite volume in a flask. The flask was inverted and shaken gently to mix thoroughly. The particle size of so-formed microemulsion was determined by photo correlation spectroscopy instrument (Marlven, U.K.) at 25 °C.

SMEDDS (500 μl) was diluted with water (5 ml) in a volumetric flask and gently mixed by inverting the flask. The droplet morphology was observed using transmission electron microscopy (JEM-2010, Japan). One drop of diluted samples was deposited on a film-coated 200-mesh copper specimen grid and allowed to stand for 10 min after which any excess fluid was removed with filter paper. The grid was later stained with one drop of 3% phosphotungstic acid (PTA) and allowed to dry for 5 min before examination

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under the electron microscope.[24)]

Dissolution Experiment Dissolution tests were performed with a dissolution apparatus (ZRS-8G, Tianjin University Electronics Co., Ltd., China). The dissolution medium was 900 ml of pH 6.8 phosphate buffer and 0.1 mol/l HCl, under the rotation speed of 50 rpm at 37±0.5 °C. To determine the amount of daidzein dissolution from the test preparations, 0.5 ml of the medium was removed for analysis at the predetermined time and replaced with fresh dissolution medium. Concentration of daidzein was analyzed by HPLC system.

Pharmacokinetic Study Male Sprague-Dawley rats were fasted overnight with free access to water before drug administration. The experiments were carried out in accordance with the guidelines of Animal Ethics Committee at Shanghai Jiao Tong University. Daidzein was suspended in 20% PEG400 solution as a control sample, the optimized self-microemulsifying formulation and control sample were administered orally (10 mg/kg of rat) with a blunt needle *via* the esophagus into stomach. Subsequently, blood samples (200 μl) were collected into heparinized micro-centrifuge tubes from caudal vein at a designated time. Blood samples were centrifuged for 5 min at 5000×$\boldsymbol{g}$ and the daidzein concentrations in the plasma were determined by HPLC. The area under the plasma concentration time curve (*AUC*) from time zero to final sampling time (12 h) was calculated by the linear trapezoidal rule. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were observed values from the experimental data.

Determination of Drugs by RP-HPLC 100 μl plasma was mixed with 200 μl acetonitrile in a tube, the tube were vortexed for 1 min and centrifuged at 5000 $\boldsymbol{g}$ for 10 min, and 20 μl of the resulting supernatant was injected into the HPLC system.

HPLC was conducted on Waters (Waters Corp., MA, U.S.A.) equipped with 1525 controller pumps, Waters 2487 detector and configured to Millennium 3.2 software. Sample was loaded onto the column by means of 717plus autosampler (Waters Corp., MA, U.S.A.). The analytical column was a reversed-phase C_{18} column (150×4.6 mm i.d., particle size 5 μm; ELITE, Dalian, People's Republic of China). The mobile phase was methanol in distilled deionized water (55 : 45, v/v) at a flow rate 1.0 ml/min. The analytes were detected using a UV detector at 260 nm wavelength.

Statistical Analysis Statistical evaluations were performed by Student *t*-test of the paired observations to analyze the different concentrations of daidzein. $p<0.05$ were considered to indicate significant differences. Data are expressed as means±S.D.

Results

Solubility Studies The actual solubility data of the daidzein is 8.215 μg/ml (in water), 7.853 μg/ml (in acidic water). To develop self-microemulsion formulations for oral delivery of poorly water-soluble daidzein, the optimum oil, surfactant and cosurfactant need to be chosen. The solubility of daidzein in various vehicles is presented in Table 1. The solubility of daidzein was highest in ethyl oleate, followed by oleic acid, castol oil and IPM. Daidzein had a higher solubility in Cremophor RH40 followed by Cremophor EL and Tween-80; daidzein also had a higher solubility in PEG400 followed by ethanol and propylene glycol. So ethyl oleate, Cremophor RH40 and PEG400 were subsequently used as the oil phase, surfactants and cosurfactant for the formulations of microemulsion containing daidzein in this study.

Pseudo-Ternary Phase Diagram Study Pseudo-ternary phase diagrams were constructed, as described in Experimental, by titration with water of mixtures of each of the selected oils with different surfactant/co-surfactant ratios, in order to find the optimal component concentration range to obtain transparent and stable O/W microemulsions. The shaded areas in the pseudo-ternary phase-diagrams were shown in Fig. 1.

The efficiency of microemulsification was good when the S/CoS concentration was more than 40% of SMEDDS formulation. It was observed that increasing the concentration of the surfactant such as Cremophor RH40 in SMEDDS formulation increased the spontaneity of the self-microemulsification region. However, the region of the self-microemulsify-

Table 1. Solubility of Daidzein in Various Vehicles

Vehicle	Solubility of daidzein (mg/ml), mean±S.D.	Vehicle	Solubility of daidzein (mg/ml), mean±S.D.
IPM	0.121±0.017	Cremophor EL	7.580±0.215
Castol oil	0.598±0.033	Cremophor RH40	9.882±0.353
Oleic acid	1.253±0.082	Ethanol	4.852±0.112
Ethyl oleate	2.327±0.106	Propylene glycol	3.147±0.126
Tween 80	5.102±0.169	PEG400	8.403±0.321

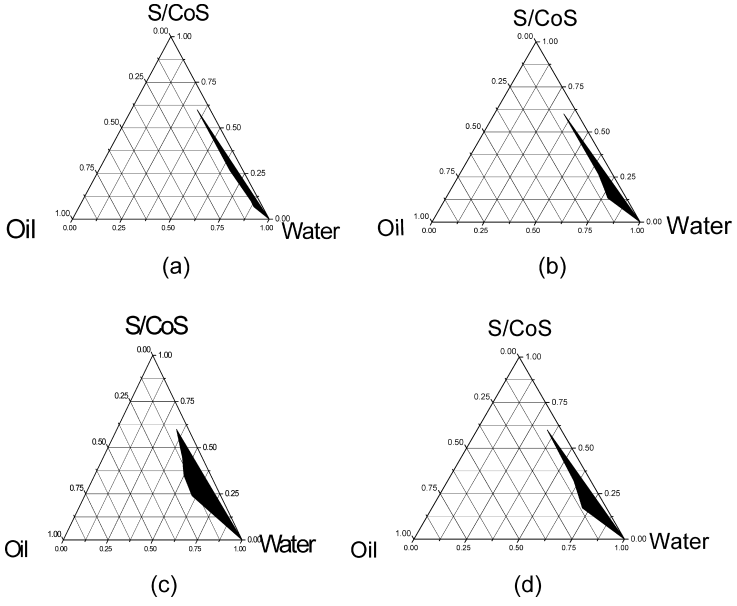

Fig. 1. Pseudo-Ternary Phase Diagrams Indicating the Efficient Self-Microemulsifying Region

S/CoS=1 : 2 (w/w) (a), 1 : 1 (w/w) (b), 2 : 1 (w/w) (c), and 3 : 1 (w/w) (d); the black area represents o/w microemulsion existence range, the white area represents coarse emulsion range.

Table 2. Composition of SMEDDS Formulations

Vehicle	Form			
	A	B	C	D
Daidzein	0.1	0.1	0.1	0.1
Ethyl oleate	0.4	—	—	0.4
IPM	—	0.4	0.4	—
Cremophor RH40	2.4	2.4	2.4	—
Cremophor EL	—	—	—	2.4
PEG400	1.2	1.2	—	—
Propylene glycol	—	—	1.2	1.2
Mean particle size (nm)	37	77	88	121

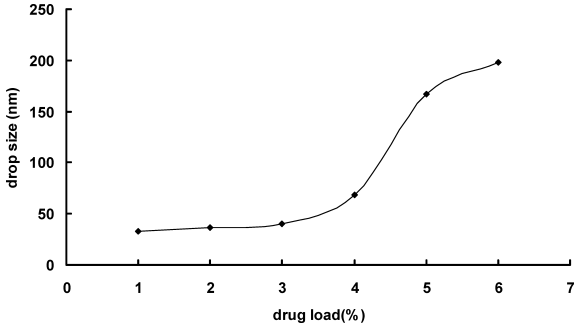

Fig. 2. Effect of the Drug Concentration on the Droplet Size

ing from the ratio of S/CoS=2 : 1 to S/CoS=3 : 1 (w/w) was decreased. So, the ratio of S/CoS=2 : 1 was chosen from the formulation.

Microemulsion Droplet Size Analysis and Morphology Observation by TEM Particle size after microemulsification was the most important property of SMEDDS, we studied the effect of several formulations on particle size. In these studies, as shown in Table 2, the average droplet size of the daidzein-containing SMEDDS was in the range of 37—121 nm. In case of the formulation of SMEDDS containing ethyl oleate as oil, cremophor RH40 as surfactant, and PEG400 as cosurfactant, the mean droplet size was smaller than the other formulations. The optimized formulation was developed and its bioavailability was compared with the control.

The effect of drug loading on particle size in distilled water is presented in Fig. 2. The mean size increased slightly with increased drug loading concentration from 1 to 3%. When drug loading further increased, particle size increased dramatically, even to as high as 100 nm or over, which was beyond the range of colloidal system and no more presented the properties of microemulsion. It could be thought that undissolved drug in the formulation affected the mean droplet size to increase.

Morphology of daidzein microemulsion was characterized using TEM (Fig. 3). It showed the spherical shape and uniform droplet size of microemulsion. In addition, the average droplet size of microemulsion increased with the amount of daidzein. This is because daidzein might embed in the interfacial film.[25] Samples were diluted with distilled water before testing to avoid multiscattering phenomena. The droplet size of the diluted microemulsion was not significantly changed.

***In Vitro* Dissolution Study** The dissolution of daidzein

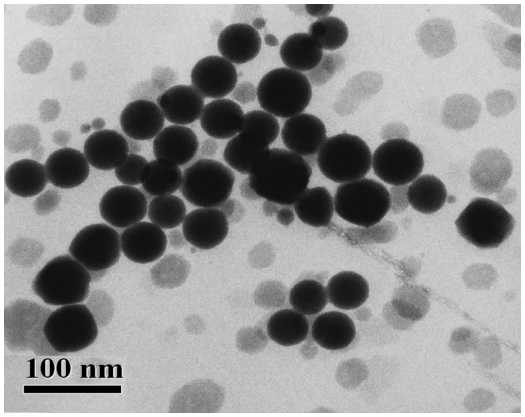

Fig. 3. Microphotograph of Daidzein Nanoemulsion by TEM (300000×)

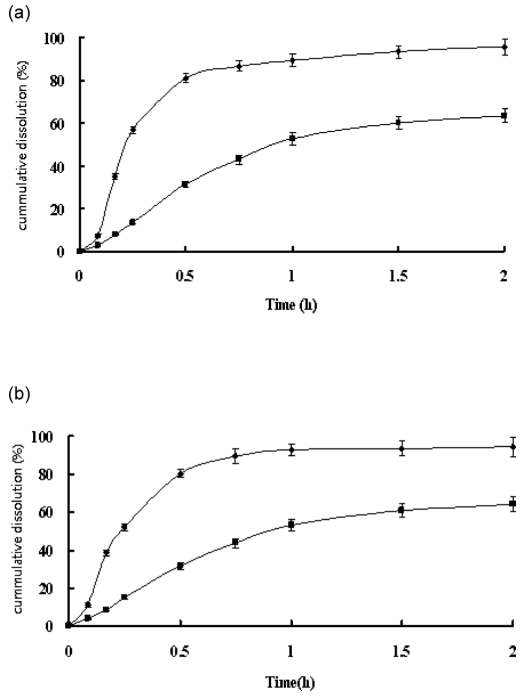

Fig. 4. Dissolution Profiles of Daidzein from SMEDDS (—●—) and Conventional Tablet (—■—) Formulation in Various Dissolution Media at 37 °C, (a) pH 6.8 Phosphate Buffer; (b) 0.1 mol/l HCl

Each point represents the mean±S.D. (n=6).

from the SMEDDS and the conventional tablet of daidzein were evaluated in pH 6.8 phosphate buffer and 0.1 mol/l HCl; the dissolution percentage of daidzein from the SMEDDS form was significantly higher than that of daidzein from the conventional tablet in both two dissolution media studied (Fig. 4). When pH 6.8 phosphate buffer and 0.1 mol/l HCl were used as the media, the percentage dissolution of daidzein from SMEDDS at 30 min was 81.2±0.8% and 80.3±1.3%, respectively. No statistically significant differences were observed among the two different dissolution media (p>0.05).

Bioavailability Study An *in vivo* absorption study was undertaken to determine whether or not the enhanced solubility and *in vitro* dissolution of daidzein in a SMEDDS could increase the GI absorption of drug after oral administration. The plasma profiles of daidzein in rats following oral

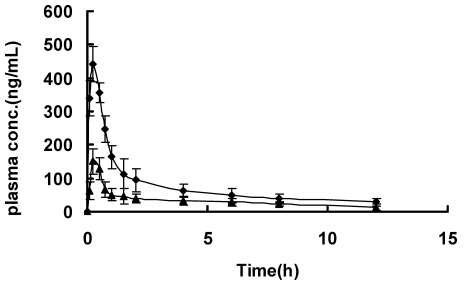

Fig. 5. Plasma Concentration–Time Profiles of Daidzein in Rats after Oral Administration (10 mg/ml) of Control Group (—▲—) and Daidzein SMEDDS (—◆—)

Data points represent mean and error bars show S.D. ($n=4$).

Table 3. Pharmacokinetic Parameters of Daidzein after Oral (10 mg/kg) Administration in Rats

	Control group	Daidzein SMEDDS
T_{max} (h)	0.38±0.14	0.27±0.17
C_{max} (ng/ml)	170.72±15.91	444.37±49.48*
$T_{1/2}$ (h)	2.99±1.49	2.65±0.67
$AUC_{0—12}$ (ng/ml·min)	380.98±67.59	954.32±158.30*
MRT (h)	4.34±0.34	3.32±0.42

Each value represents the mean±S.D. of at least four experiments. $*p<0.05$, compared with control.

administration of the control group and SMEDDS form were compared. Figure 5 showed that plasma concentration profiles of daidzein for SMEDDS represented significantly greater improvement of drug absorption than the control group. Pharmacokinetic parameters of the maximum plasma concentration (C_{max}) and the corresponding time (T_{max}) for daidzein following oral administration were shown in Table 3. The area under the concentration–time curve ($AUC_{0\to12\,h}$) was estimated according to the linear trapezoidal rule.

In pharmacokinetic parameters of SMEDDS form, $AUC_{0\to12\,h}$ and C_{max} were 954.32±158.30 ng/ml·min and 444.37±49.48 ng/ml, respectively, compared with the control group which were 380.98±67.59 ng/ml·min and 170.72±15.91 ng/ml, respectively. SMEDDS enhanced the values of $AUC_{0\to12\,h}$ and C_{max} of drug compared with the control group.

Discussion

Microemulsion technology is one of the important drug delivery principles, which improves the absorption of poorly absorbable drugs. Microemulsions are defined in general as thermodynamically stable, isotropically clear dispersions,[11,12] which are known to enhance the water solubility of hydrophobic compounds and attain their better absorption. One of the advantages of self-microemulsion system is that the mixture of drug and emulsifier can form fine microemulsion with only gentle agitation as soon as the formulation immingles with intestinal fluid. This property makes self-microemulsion a good vehicle for the oral delivery of poorly absorbable drugs.

The ternary phase diagrams (Fig. 1) show that much higher concentration of surfactant, much higher self-microemulsifying region in phase diagrams. The relatively high concentration of surfactant required in this study to form microemulsions is in accordance with other studies, where the use of high concentrations of surfactants was found to be necessary to achieve fast and effcient self-microemulsication.[12,26]

A good relation between the visual observations and the achieved droplet sizes was observed. In the microemulsion area clear to bluish transparent microemulsions with droplet sizes less than 100 nm were formed compared with white emulsion-like dispersions with droplet sizes above 100 nm outside this area, indicating that if microemulsions are formed the resulting droplet size is very small due to the thermodynamical stability of these systems.[27]

In vitro dissolution study, the dissolution percentage of daidzein from the SMEDDS form was significantly higher than that of daidzein from the conventional tablet in two dissolution media. It could suggest that daidzein dissolved perfectly in SMEDDS form could be dissolution due to the small droplet size, which permits a faster rate of drug dissolution into aqueous phase, faster than conventional tablet, and it could affect the bioavailability. The results also showed that the developed formulation was not affected by the pH and ionic strength of the dissolution media over the pH range 1.0—6.8.

Figure 5 showed that plasma concentration profiles of daidzein for SMEDDS form represented significantly greater improvement of drug absorption than the control group. SMEDDS might be a promising approach for the effective absorption into oral administration delivery of daidzein and could increase bioavailability for the other poorly water-soluble drug. The SMEDDS were expected to give rise to a higher bioavailability due to more rapid and uniform distribution of the drug substance in the GI.[28] The large surface area obtained after administration of the SMEDDS promotes a rapid dissolution of the drug substance[29] and/or the drug substance may be absorbed directly from the small droplets of the microemulsion.[27,30]

In conclusion, the optimal formulation of SMEDDS containing daidzein (high drug loading and small particle size) was as following: Ethyl oleate (10%), Cremophor RH 40 (60%), and PEG400 (30%) due to high affinity for the continuous phase and forming the smallest particle size. *In vitro* dissolution studies revealed that dissolution of daidzein from SMEDDS was faster than the conventional tablet. *In vivo* studies for clinical purpose, SMEDDS showed significantly greater extent of absorption than the control group. Our studies illustrated the potential use of SMEDDS for the delivery of hydrophobic compounds, such as daidzein by the oral route.

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