International Journal of Pharmaceutics 371 (2009) 148–155

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems

Jing Cui[a,b], Bo Yu[c,d], Yu Zhao[e], Weiwei Zhu[a], Houli Li[a], Hongxiang Lou[f], Guangxi Zhai[a,*]

[a] Department of Pharmaceutics, School of Pharmaceutical Sciences, Shandong University, 44 West Wenhua Road, Jinan 250012, PR China
[b] Department of Pharmacy, Qilu Hospital, Shandong University, Jinan 250012, PR China
[c] Center for Affordable Nanoengineering of Polymeric Biomedical Devices (CANPBD), a NSF Nanoscale Science and Engineering Center, the Ohio State University, Columbus, OH 43210, USA
[d] Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, OH 43210, USA
[e] Department of Pharmacognosy, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, PR China
[f] Department of Natural Product Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, PR China

ARTICLE INFO

Article history:
Received 27 July 2008
Received in revised form 4 December 2008
Accepted 6 December 2008
Available online 13 December 2008

Keywords:
Curcumin
Self-microemulsifying drug delivery system (SMEDDS)
Simplex lattice experiment design
Microemulsion

ABSTRACT

Curcumin is a poorly water-soluble drug and its oral bioavailability is very low. A new self-microemulsifying drug delivery system (SMEDDS) has been successfully developed to improve the solubility and oral absorption of curcumin. Suitable compositions of SMEDDS formulation were screened *via* solubility studies of curcumin and compatibility tests. The formulation of curcumin-loaded SMEDDS was optimized by a simplex lattice experiment design. The optimal formulation of SMEDDS was comprised of 57.5% surfactant (emulsifier OP:Cremorphor EL = 1:1), 30.0% co-surfactant (PEG 400) and 12.5% oil (ethyl oleate). The solubility of curcumin (21 mg/g) significantly increased in SMEDDS. The average particle size of SMEDDS-containing curcumin was about 21 nm when diluted in water. No significant variations in particle size and curcumin content in SMEDDS were observed over a period of 3 months at 4 °C. The spherical shape of microemulsion droplet was observed under TEM. The dissolution study *in vitro* showed that more than 95% of curcumin in SMEDDS could be dissolved in pH 1.2 or pH 6.8 buffer solutions in 20 min, however, less than 2% for crude curcumin in 60 min.The *in situ* absorption property of curcumin-loaded SMEDDS was evaluated in intestines of rats. The results showed the absorption of curcumin in SMEDDS was *via* passive transfer by diffusion across the lipid membranes. The results of oral absorption experiment in mice showed that SMEDDS could significantly increase the oral absorption of curcumin compared with its suspension. Our study illustrated that the developed SMEDDS formulation held great potential as a possible alternative to traditional oral formulations of curcumin.

1. Introduction

Curcumin, a naturally active constituent extracted from the plants of the *Curcuma longa*, its structure shown in Fig. 1, has a variety of biological activities and pharmacological actions, such as anti-tumor, anti-inflammatory, anti-virus, anti-oxidation and anti-HIV, and low toxicity with promising clinical application (Araujo and Leon, 2001; Hsu and Cheng, 2007). However, curcumin is slightly absorbed in the gastrointestinal tract due to its poor solubility in water (the maximum solubility was reported to be 11 ng/ml in plain aqueous buffer pH 5.0) (Tonnesen et al., 2002). The oral bioavailability of curcium is very low (only 1% in rats) (Pan et al., 1999; Yang et al., 2007), in clinical trial quantifiable serum levels were not achieved until doses of up to 3.6 g were used (Johnson and Mukhtar, 2007). Therefore, the dose of oral curcumin at 3.6 g daily was recommended for phase II clinical trial of treating advanced colorectal cancer (Sharma et al., 2004).

To improve the bioavailability of curcumin, numerous approaches have been investigated. These approaches include loading curcumin into liposomes or nanoparticles, forming curcumin–phospholipid complex and synthesizing structural analogues of curcumin (Marczylo et al., 2007; Anand et al., 2007).

Self-microemulsifying drug delivery system (SMEDDS) has recently emerged as one of the most interesting approaches to improve the solubility, dissolution and oral absorption for poorly water-soluble drugs (Zhang et al., 2008). SMEDDS is an isotropic mixture of oil, surfactant, co-surfactant and drug substance, which can form a microemulsion under conditions of gastrointestinal fluid and gastrointestinal motility after oral administration. The resultant microemulsion with a particle size less than 100 nm and the increasing solubility of hydrophobic drug can enhance the absorption in gastrointestinal tract (Patel and Sawant, 2007). Cyclosporine

* Corresponding author. Tel.: +86 531 8838 2015; fax: +86 531 8838 2731.
E-mail address: zhaiguangxi@yahoo.com.cn (G. Zhai).

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doi:10.1016/j.ijpharm.2008.12.009

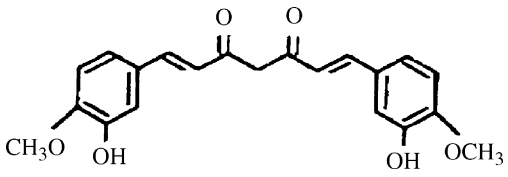


Fig. 1. The structure of curcumin.

A has been prepared in the form of SMEDDS and commercially available as Neoral®, triggers much more attention on SMEDDS. Many poorly water-soluble drugs such as acyclovir, α-Asarone, atorvastatin, and fenofibrate have been reported to improve their oral bioavailability by SMEDDS (Wang et al., 2006; Shen and Zhong, 2006; Patel and Vavia, 2007), but curcumin formulated in SMEDDS has not been evaluated yet so far.

The objectives of our present study were to design, prepare, and characterize a SMEDDS formulation of curcumin, and assess its *in situ* absorption property in rat intestine and *in vivo* oral absorption in mice. The solubility of curcumin was determined in various vehicles. SMEDDS formulations were tested for microemulsifying properties, and the resultant formulations loaded with curcumin were optimized by a simplex lattice experiment design. The optimal formulation of curcumin was further investigated for physicochemical characteristics, *in situ* absorption property in rat intestines as well as *in vivo* oral absorption in mice.

2. Materials and methods

2.1. Materials

Curcumin, propylene glycol, poloxamer 188 and Cremorphor EL were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Emulsifier OP was obtained from Guangcheng chemical agent Co., Ltd. (Tianjin, China). Ethyl oleate and PEG 400 were purchased from chemical agent Co., Ltd. (Shanghai, China). Peanut oil and Tween 80 were purchased from Zhonghua chemical engineering Co., Ltd. (Shanghai, China). Methanol of high-performance liquid chromatography (HPLC) grade was purchased from Tianjin SiYou chemical agent Co., Ltd. (Tianjin, China). All other chemicals were reagent grade.

2.2. Screening of SMEDDS formulation

2.2.1. Solubility studies

To find out appropriate oils and surfactants as compositions of SMEDDS, the solubility of curcumin in various oils such as peanut oil, castor oil, ethyl oleate, and paraffin oil and various surfactants including emulsifier OP, Tween 80, Cremorphor EL-40 and poloxamer 188 were determined. An excess amount of curcumin was added to 5 ml of oil or 20% (w/w) surfactant aqueous solutions. The resultant mixture was shaken reciprocally at 37 °C for 72 h, followed by centrifugation at 12000 rpm for 10 min. The supernatant was filtered through a membrane filter (0.45 μm) to remove the remaining insoluble curcumin. After the appropriate dilution with methanol, the drug concentration in the filtrate was quantified by HPLC.

2.2.2. Compatibility tests

To assess the compatibility between surfactant solution and oil, ethyl oleate as the chosen oil was dropped into 1 ml surfactant solution or mixed surfactant solutions, and then the resulting mixture was mixed for 5 min using a vortex mixer. The added amount of ethyl oleate and the appearance of resulting solutions were recorded. Based on the properties of microemulsion, the clearance and transparency (visual test) of resulting solution were used as standards to evaluate compatibility of oil and surfactant solution (Kawakami et al., 2002). Under the conditions of clearance and transparency of resulting solution, more amount of added ethyl oleate showed better compatibility with surfactant or mixed surfactant solutions.

Similarly, co-surfactants were screened on the basis of their compatibility with the mixed system of the selected oil and surfactant solutions. A series of co-surfactants such as alcohol, isopropyl alcohol, 1,2-propylene glycol, glycerol and PEG 400 were respectively dropped to the emulsion composed of mixture of emulsifier OP:EL-40 (1:1, w/w), ethyl oleate and water at the weight ratio of 1:0.3:5. The added amounts of co-surfactants were recorded at two phase-change points, i.e. the whole system becoming clear from being turbid, and becoming turbid again from being clear. Under the conditions of keeping the system clear and transparent, the less amount and the larger range between two phase-change points of added co-surfactant showed the better compatibility with the mixed system of oil and surfactant solution (Kale and Allen, 1989).

2.3. Construction of phase diagrams

Pseudoternary phase diagrams were constructed in order to obtain the concentration range of components for the existing region of microemulsions. The weight ratio of surfactant to co-surfactant (*Km*) was varied as 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. For each pseudoternary phase diagram at a specific surfactant/co-surfactant weight ratio, the oily mixtures of oil, surfactant and co-surfactant were prepared with the weight ratio of oil to the mixture of surfactant and co-surfactant at 5:95, 10:90, 15:85, 20:80, 25:75, 30:70, 35:65, 40:60, 45:55, 50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 85:15, 90:10, and 95:5, respectively. Water was added drop by drop to each oily mixture under proper magnetic stirring at 37 °C until the mixture became clear at a certain point. The concentrations of the components were recorded in order to complete the pseudoternary phase diagrams, and then the contents of oil, surfactant, co-surfactant and water at appropriate weight ratios were selected based on these results.

2.4. Preparations of SMEDDS formulations

SMEDDS was prepared according to a recently reported method (Patel and Vavia, 2007; Holm et al., 2006). Variable proportions of oil, surfactant and co-surfactant were added into a 10 ml screw-capped glass tube, and the components were mixed by gentle stirring. After complete dissolution, SMEDDS, a clear and transparent solution, was obtained. Based on the results of above experiment and the reported concentration scope of three ingredients forming SMEDDS (Holm et al., 2006; Chen et al., 2008; Cirri et al., 2007), the contents of surfactant, co-surfactant and oil were chosen at the range of 30–65%, 30–65% and 5–40%, respectively, in order to obtain the optimal formulation of SMEDDS.

2.5. Droplet size and solubility studies of curcumin in SMEDDS

One gram of SMEDDS was diluted in 200 ml-distilled water. The droplet size/distribution of the prepared solution was determined with N5 Submicron particle size Analyzer (Beckman Coulter, UK) which has a detection range from 2 to 5000 nm. Each sample was analyzed in triplicate.

Excessive curcumin was added into 1 g of SMEDDS, and the resultant suspension was shaken at 37 °C for 24 h using an oscillator (THZ-82, Changsi Commercial Ltd., China). Then the suspension was centrifuged at 12000 rpm for 10 min. 0.1 g of supernatant was taken out and diluted with 50% ethanol for further analysis. The solubility of curcumin in SMEDDS was obtained by absorption determination

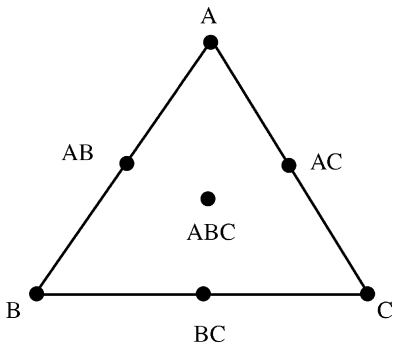


Fig. 2. Equilateral triangle representing simplex lattice design for three components.

of the supernatant at 432 nm using a spectrophotometer (UV-2102, Shanghai Instrument Ltd., China).

2.6. Formulation optimization of curcumin-loaded SMEDDS

A simplex lattice experiment design was used to optimize the composition of SMEDDS (Subramanian et al., 2004). In this design, three factors were evaluated by changing concentrations of components simultaneously and keeping the total concentration constant. As shown in Fig. 2, the simplex lattice design for a three-component system was represented by an equilateral triangle in two-dimensional space. Seven batches of SMEDDS carrying curcumin were prepared, including three vertexes (A, B, C), three half-way points between vertices (AB, BC, AC), and one center point (ABC). The concentrations of surfactant, co-surfactant and oil were selected as independent variables. The solubility of curcumin in SMEDDS and the mean particle size of microemulsification from SMEDDS were taken as responses.

The responses for seven formulations were used to fit an equation for simplex lattice model (Mao et al., 2004; Patel et al., 2007), which can predict the properties of all possible formulations. Graphs of these properties in the form of contour plots were constructed by Matlab Software (Kompany-zareh and Khoshkam, 2008; Casanova et al., 2007). With the aid of Matlab Software, the model equation was developed as the best representation of the relationship between the solubility or particle size and the measured characteristics.

2.7. Characterization of SMEDDS-containing curcumin

2.7.1. Morphology

The morphology of SMEDDS was observed using a transmission electron microscope (TEM) (JEM-1200EX, JEOL, Tokyo, Japan). SMEDDS was diluted with distilled water at 1:200 and mixed by slightly shaking. One drop of diluted samples was deposited on a film-coated copper grid and then stained with one drop of 2% aqueous solution of phosphotungstic acid (PTA), allowing to dry before observation under the electron microscope.

2.7.2. Electrical conductivity and refractive indices

Electrical conductivity of formed microemulsion from SMEDDS was measured using a conductivity meter (DDS-11C, Shanghai Instrument, China). Based on electrical conductivity, the phase systems of the microemulsion were determined. Refractive indices of formed microemulsion by diluting SMEDDS with distilled water at 1:200 were measured with a thermostated Abbe refractometer (Shijiazhuang Optical Instrument Factory, China).

2.7.3. Stability studies

In order to evaluate the stability of the optimized SMEDDS-containing curcumin, it was added into sealed glass vials and then these vials were stored at 4 °C for 3 months, then the clarity, phase separation, concentration of curcumin and particle size after dilution with distilled water at 1:200 were investigated at predetermined intervals.

2.7.4. Effects of various diluents on microemulsification

In order to determine the effects of different media on the particle size of microemulsification, 1 g of SMEDDS was added into 200 ml of distilled water, phosphate buffered saline (pH 6.8) and 0.1 mol/l hydrochloride solution, respectively, and the resulted solutions were slightly shaken. The particle sizes of these solutions were analyzed with the method described in Section 2.5.

2.8. Dissolution studies

In order to compare the dissolution behaviors of curcumin-loaded SMEDDS and crude curcumin, pH 1.2 or pH 6.8 buffer solutions was chosen as dissolution medium according to the reported study (Paradkar et al., 2004; Borhade et al., 2008). Dissolution studies were performed according to the previously described method (Patel and Vavia, 2007; Borhade et al., 2008). SMEDDS containing 20 mg of curcumin or 20 mg of crude curcumin was filled in hard gelatin capsules and introduced into 500 ml of a dissolution medium and maintained at 37 °C. The revolution speed of the paddle was kept constant at 100 rpm. The aliquot of 5 ml was withdrawn at 0, 10, 20, 30, 50 and 60 min, and filtered through 0.45 μm membrane filters. The concentration of curcumin was determined spectrophotometrically at 432 nm. The removed volume was replaced each time with 5 ml of fresh medium.

2.9. In situ absorption evaluation of curcumin-loaded SMEDDS in rat intestine

Male Wistar rats weighing 200 ± 20 g (SCXK (Lu) 20030004) were obtained from Experimental Animal Center of Shandong University, China. All animal experiments complied with the requirements of the National Act of the People's Republic of China on the use of experimental animals. Animals were acclimated for at least 5 d before the experiments and housed in cages (5 each) under constant temperature (22 ± 2 °C) with free access to food and drinking water.

The absorption property of loaded curcumin SMEDDS was investigated with the established *in situ* intestinal perfusion methods in rats (Cirri et al., 2007; Zhou et al., 2008; Song et al., 2006; Zakeri-Milania et al., 2007; Liao et al., 2005). Briefly, rats were fasted for 12 h before experiment with free access to water. The rats after anaesthetized were placed under an infrared lamp to keep normal body temperature. The whole small intestine was surgically exposed and ligated for perfusion and cannulated with plastic tubing (diameter 0.4 cm). The cannulated segment was rinsed with 37 °C normal saline and attached to the perfusion assembly which consisted of a peristaltic pump (BT00-100M, Baoding Longer Precision Pump Co., Ltd., China) and a 100 ml volumetric cylinder containing 100 ml sample solution. Care was taken to handle the small intestine gently in order to maintain an intact blood supply. The entire surgical area was covered with a piece of sterilized absorbent gauze wetted with normal saline. At the beginning of the test, sample solution was perfused through the intestine at a flow rate of 5 ml min^{-1}. Ten minutes later, the volume of perfusion solution in the circulation system as the 0 min volume was recorded and the flow rate was adjusted to 2.5 ml min^{-1}. During 6 h perfusion period, at predetermined time interval, 1 ml of sample solution was taken out, the volume of solution in the circulation system was recorded, and then 2 ml Krebs-Rings solution was added in. Samples were frozen immediately and stored at −20 °C until analysis. Before analysis, samples were fused at 25 °C and mixed with 9 ml

methanol, and the resulting solution was centrifuged at 12000 rpm for 10 min, 20 μl of supernatant was introduced into HPLC.

The absorption constant (*Ka*) is calculated using Fick's equation: $Ka = [-\ln(X/X_0)]/t$, where X_0 is the amount of drug before absorption, X is the residue amount of drug after absorption. *Ka* can be obtained as the slope from the regression curve of $-\ln(X/X_0)$ *versus* time, and $t_{1/2}$ can be obtained when X is equal to $X_0/2$ (Chen et al., 2008).

The perfusion solution was Krebs-Rings buffer solution containing 7.8 g NaCl, 0.35 g KCl, 1.37 g $NaHCO_3$, 0.02 g $MgCl_2$, 0.22 g NaH_2PO_4 and 1.48 g glucose in 1000 ml purified water.

Quantities of curcumin-loaded SMEDDS were in Krebs-Rings buffer to produce different concentrations of test solutions at a curcumin dose of 25, 50 and 100 mg kg^{-1}, respectively.

2.10. In vivo absorption of curcumin-loaded SMEDDS in mice

Absorption studies *in vivo* were performed in male Kunming mice with 20 ± 2 g body weight based on the established method (Wahlstrom and Blennow, 1978). The mice were fasted overnight before experiment with free access to water. Curcumin suspension (curcumin dispersed in 0.4% sodium carboxymethycellulose, CMC-Na solution) and curcumin-loaded SMEDDS (50 mg/kg of body weight) were given to mice by intragastric administration, respectively. The mice were sacrificed at 2, 4, 6, 8, 10, 12 and 24 h after administration, respectively. The content from lower esophageal to anus in tested mice was taken out immediately and the mucous membrane was washed with methanol, the content and washed methanol were mingled with the egesta collected in the whole experiment. About 80 ml of methanol was added into the above mixture, and the resulted samples were subjected to supersonic for 1 h and filtrated. The residue was washed with methanol for two times, and the washed methanol was filtrated. And then all the filtrate was merged together and made to 100 ml with methanol. The resulted solution was filtrated with 0.45 μm membrane filter before injection onto HPLC for curcumin analysis. The absorbed amount of curcumin by mice was calculated by the administrated amount (x_1) minus the amount of curcumin both being existed in the gastrointestinal tract and being excreted from the feces (x_2). The absorption percentage (x_{ab}%) could be achieved by the following equation:

$$x_{ab}\% = \frac{x_1 - x_2}{x_1} \times 100\%$$

2.11. HPLC analysis of curcumin

All samples were analyzed by reverse-phase HPLC using a phenomenex-C_{18} column (5 μm, 4.6 mm × 250 mm) at room temperature. Curcumin was detected at 428 nm. The mobile phase was a mixture of methanol:H_2O (containing 3.6% glacial acetic acid) (73:27, v/v) and was delivered at a flow rate of 1 ml min^{-1}. The retention time for curcumin was about 7.5 min and linearity was obtained in the range from 0.5 to 15 μg/ml. The coefficient of variation for intra- and inter-day assays was less than 7.5%. The average recovery of curcumin from the perfusion solution was greater than 98%, and 89.8% for curcumin extracted from both whole gastrointestinal tract contents and the excreted feces.

2.12. Statistical analysis

The statistical significance of the difference between mean values was assessed by use of Student's *t*-test. Statistical probability (p) values less than 0.05 were considered significantly different.

Table 1
Solubility of curcumin in various oils and surfactant solutions (mean ± S.D.; $n = 5$).

Oil	Solubility (mg/g)	20% Surfactant solution	Solubility (mg/ml)
Paraffin oil	<0.010	Poloxamer 188	<0.010
Peanut oil	0.142 ± 0.013	Cremorphor EL-40	1.145 ± 0.115
Castor oil	0.267 ± 0.023	Tween 80	1.353 ± 0.084
Ethyl oleate	0.357 ± 0.032	Emulsifier OP	1.924 ± 0.232

3. Results

3.1. Screening of SMEDDS formulation compositions

Solubility of curcumin in various vehicles was presented in Table 1. Among four tested oils, ethyl oleate showed the best solubility for curcumin. Hence, ethyl oleate was chosen as oil phase in SMEDDS of curcumin. In tested surfactants, emulsifier OP had the best solubility for curcumin, followed by Tween 80 and Cremorphor EL-40. In order to find out a surfactant or a mixture of surfactants with the best compatibility with oil, solubility tests of ethyl oleate in emulsifier OP, the mixture of emulsifier OP and Cremorphor EL-40 or emulsifier OP and Tween 80 at the ratio of 2:1, 1:1 and 1:2, respectively, were performed. As shown in Table 2, ethyl oleate gave the highest solubility in the mixed surfactants consisting of emulsifier OP:EL-40 = 1:1 (w/w). Consequently, the mixture of emulsifier OP:EL-40 (1:1) was used as the desirable surfactant.

Based on the above findings, 1 g emulsion composed of emulsifier OP:EL-40 (1:1, w/w), ethyl oleate and water at the weight ratio of 1:0.3:5 was applied to further assess the best co-surfactant. It was found that PEG 400 could form clear and transparent microemulsion solution with less amount and a larger range from 0.09 to 0.288 g with the above emulsion than that of glycerol from 0.107 to 0.285 g. Other co-surfactants such as alcohol, isopropyl alcohol, 1,2-propylene glycol cannot form microemulsion at the dose of less than 0.5 g under the same condition. Therefore, it was reasonable to select PEG 400 as the co-surfactant.

3.2. Construction of phase diagrams

The construction of pseudoternary phase diagrams was used to obtain appropriate concentration ranges of components in the areas of forming microemulsions. The pseudoternary phase diagrams of O/W microemulsions consisted of the mixture of emulsifier OP:Cremorphor EL (1:1, w/w), PEG 400, ethyl oleate and distilled water with various *Km* as described in Fig. 3. The diagrams showed that the area region of microemulsions was changed from small to large to small as *Km* decreased, reaching the maximum point at *Km* of 6:4. And all microemulsion formulations tested at various *Km* could be diluted with water without limit.

3.3. Formulation optimization of curcumin-loaded SMEDDS

Simplex lattice method was used to optimize the formulation of curcumin-loaded SMEDDS. The concentrations of surfactant (X_1), co-surfactant (X_2) and oil (X_3) were chosen as the independent

Table 2
Solubility of ethyl oleate in surfactant or mixed surfactant solutions (mean ± S.D.; $n = 5$).

20% Surfactant solutions	Solubility of ethyl oleate (g/ml)
Emulsifier OP	0.025 ± 0.003
Emulsifier OP:Tween 80 = 2:1	0.028 ± 0.003
Emulsifier OP:Tween 80 = 1:1	0.033 ± 0.004
Emulsifier OP:Tween 80 = 1:2	0.031 ± 0.004
Emulsifier OP:EL-40 = 2:1	0.041 ± 0.004
Emulsifier OP:EL-40 = 1:1	0.053 ± 0.006
Emulsifier OP:EL-40 = 1:2	0.048 ± 0.005

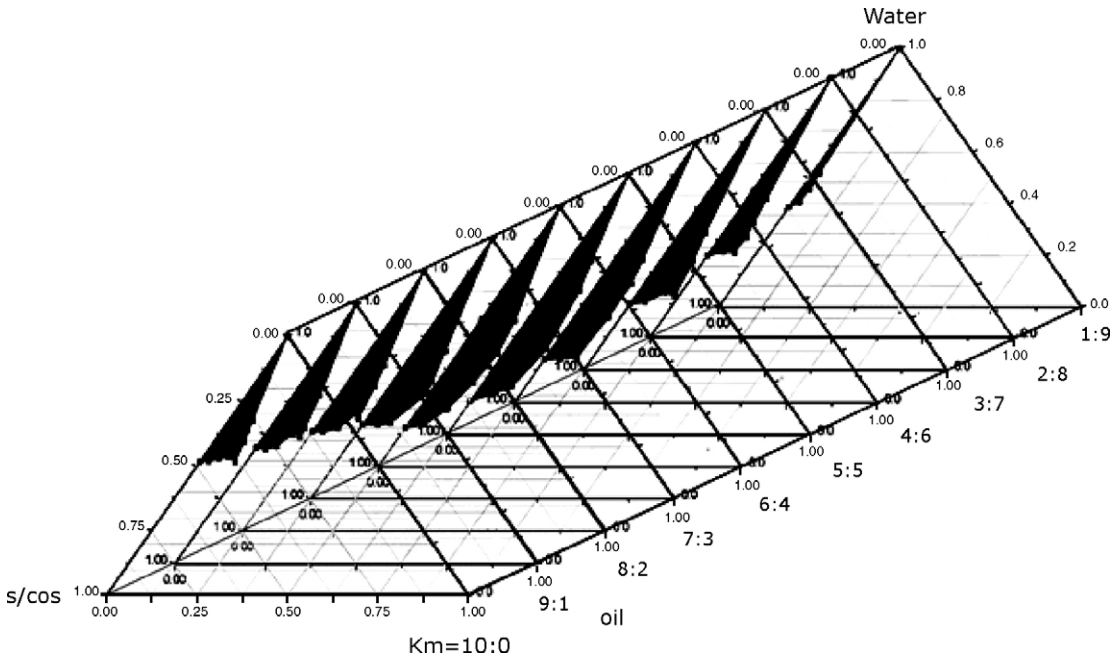


Fig. 3. Pseudoternary phase diagrams of microemulsions composed of oil (ethyl oleate), surfactant (emulsifier OP:Cremorphor EL = 1:1, w/w), co-surfactant (PEG 400) and water.

variables. The solubility of curcumin in SMEDDS and the mean particle size of formed microemulsion by diluting SMEDDS with distil water were taken as responses (Y), respectively. The equation for simplex lattice model is described as follows:

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (1)$$

where Y is the dependent variable and b_i is the estimated coefficient for the factor X_i. The major effects (X_1, X_2, and X_3) represent average results of changing one factor at a time from its low to high value, and the interactions X_1X_2, X_2X_3, X_1X_3, and $X_1X_2X_3$ show how the responses change when two or three factors change simultaneously.

According to simplex lattice model and the selected concentration ranges of surfactant, co-surfactant and oil, seven different formulations of SMEDDS carrying curcumin were constructed. The results of their solubility and mean particle size were given in Table 3.

With the help of Matlab Software, the fitted results are shown in Eqs. (2) and (3):

$$Y_{size} = -410.8333X_1 - 453.8537X_2 + 84.4737X_3 + 2.0457 \times 10^3X_1X_2 + 6.0715 \times 10^3X_1X_3 + 7.4233 \times 10^3X_2X_3 - 3.1313 \times 10^4X_1X_2X_3 \, (r = 0.9999) \quad (2)$$

Table 3
Compositions of seven formulations as per simplex lattice design and their solubility and mean particle size (mean ± S.D.; $n = 5$).

Formulation number	Formulation components (%)			Mean particle size (nm)	Solubility (mg g^{-1})
	Surfactant	Co-surfactant	Oil		
1	65	30	5	3.3	22.14 ± 0.95
2	30	65	5	11.9	13.44 ± 0.09
3	30	30	40	450.6	13.97 ± 0.61
4	47.5	47.5	5	22.3	18.12 ± 0.31
5	47.5	30	22.5	125.2	19.75 ± 0.44
6	30	47.5	22.5	170.9	14.02 ± 0.24
7	41.7	41.7	16.7	39.7	17.34 ± 0.16

$$Y_{solubility} = 25.1499X_1 + 2.5348X_2 - 23.0626X_3 + 18.2600X_1X_2 + 99.8786X_1X_3 + 55.0949X_2X_3 - 148.7110X_1X_2X_3 \, (r = 0.9999) \quad (3)$$

Eqs. (2) and (3) can be used to calculate the predicted values for other formulations in the design space. In order to get proper formulation, contour plots of mean particle size and solubility were further constructed when responses (Y) were set as certain values (Fig. 4).

In Fig. 4(A), two mean particle size curses stand for 10 and 100 nm, respectively. Trace contours were constructed when $Y_{solubility}$ was equal to 15.0, 17.5, 20, 21, 21.5 and 22 mg/g, as shown in Fig. 4(B). According to the requirements of SMEDDS about solubility and size scope, the common part between Y_{size} located at the scope of 10–100 nm and $Y_{solubility}$ located at the field of 20–22 mg/g was the optimized region for the concentrations of surfactant and co-surfactant (Fig. 4(C)). So, the composition of optimized formulation was chosen as follows: 57.5% surfactant (28.75% emulsifier OP, 28.75% Cremorphor EL), 30% co-surfactant (PEG 400) and 12.5% oil (ethyl oleate).

The chosen concentrations of surfactant, co-surfactant and oil were introduced into above Eqs. (2) and (3). As a result, mean particle size of SMEDDS and the solubility of curcumin in SMEDDS can be calculated as 30.63 nm and 21.53 mg/g, respectively. The results for above two parameters obtained by experimental determination were 31.46 nm and 21.88 mg/g. Furthermore, as shown in Fig. 5, the above optimal formulation was located in the shadow area forming SMEDDS in the pseudoternary phase diagram, which was constructed by mixing ethyl oleate, the mixture of emulsifier OP:Cremorphor EL (1:1) and PEG 400 at different ratio and recording the concentrations of components when clear and transparent solutions were formed. So it can be concluded that the simplex lattice model equation accurately predicts the experimental results.

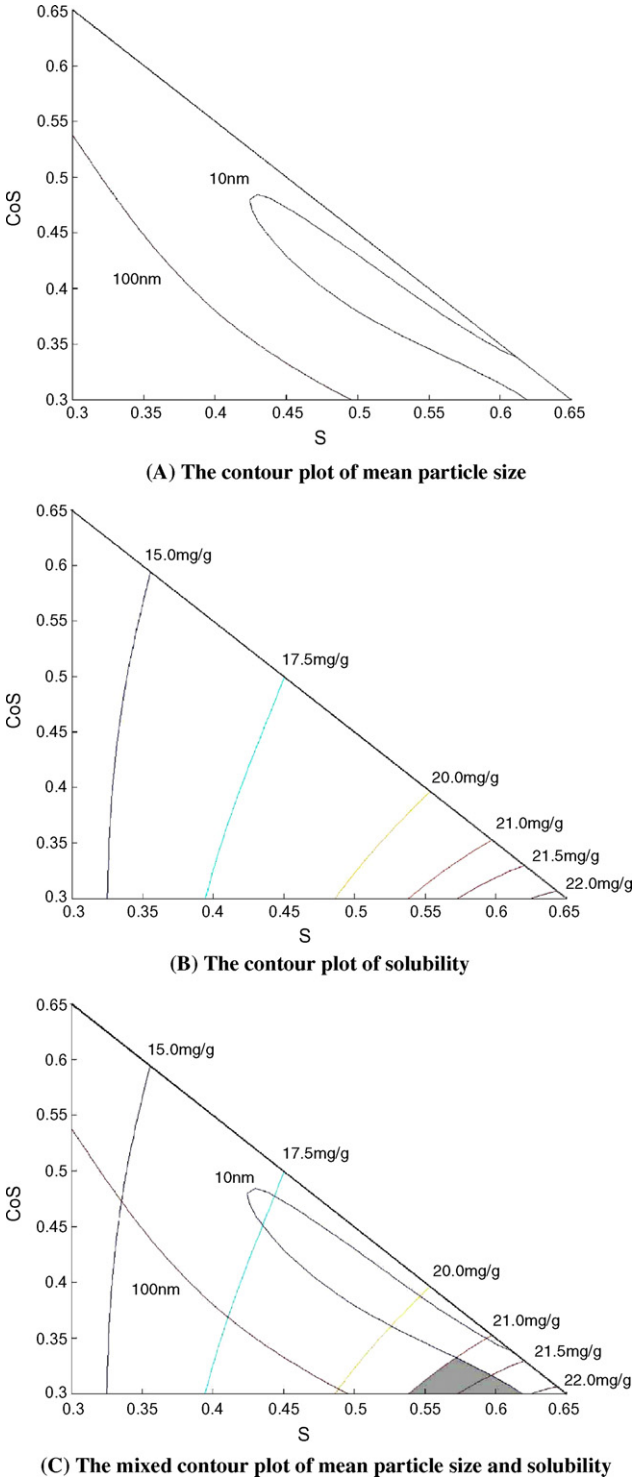
3.4. Characterization of SMEDDS-containing curcumin

Morphology of the microemulsions formed from optimized SMEDDS-containing curcumin was viewed under a TEM, the

Table 4
Parameters of microemulsification of SMEDDS (mean ± S.D.; $n = 3$).

Samples	Refractive indices	Electrical conductivity ($\mu s\,cm^{-1}$)	Zeta potential (mV)	Particle size (nm)		
				Distilled water	Hydrochloride solution (0.1 M)	PBS (pH 6.8)
A	1.34 ± 0.012	29.8 ± 1.9	−1.95 ± 0.12	31.5 ± 1.9	31.8 ± 1.7	30.7 ± 2.1
B	1.33 ± 0.014	22.4 ± 2.1	−2.13 ± 0.17	21.4 ± 1.5	22.7 ± 1.5	21.9 ± 1.3

A, blank SMEDDS; B, curcumin-loaded SMEDDS.



(A) The contour plot of mean particle size

(B) The contour plot of solubility

(C) The mixed contour plot of mean particle size and solubility

Fig. 4. The contour plots of response: (A) the contour plot of mean particle size; (B) the contour plot of solubility; (C) the mixed contour plot of mean particle size and solubility.

microemulsion vesicles appeared as perfect round shape without aggregation. The other parameters for physicochemical characters of the optimized formulation were shown in Table 4. Refractive indices and Zeta potential of microemulsification of SMEDDS were not obviously changed before and after loading curcumin. However, electrical conductivity and mean particle size were decreased significantly after loading curcumin. Based on the difference in electrical conductivity of microemulsification from SMEDDS before and after loading curcumin, it can be concluded that o/w microemulsion could be formed when SMEDDS was diluted with distilled water.

After storage at 4 °C for 3 months, the optimized SMEDDS solution containing curcumin was still clear and transparent without any phase separation. Concentration of curcumin in SMEDDS and particle size after microemulsification were not also changed significantly, in comparison to the original one. It suggested that curcumin-loaded SMEDDS was stable under above conditions.

3.5. Dissolution studies

Curcumin is practically insoluble in water at acidic or neutral pH (Tonnesen et al., 2002). As shown in Fig. 6, crude curcumin showed

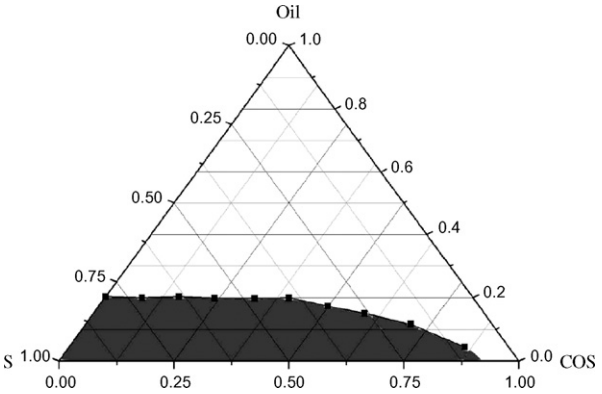


Fig. 5. Phase diagram of SMEDDS composed of oil, surfactant(s) and co-surfactant.

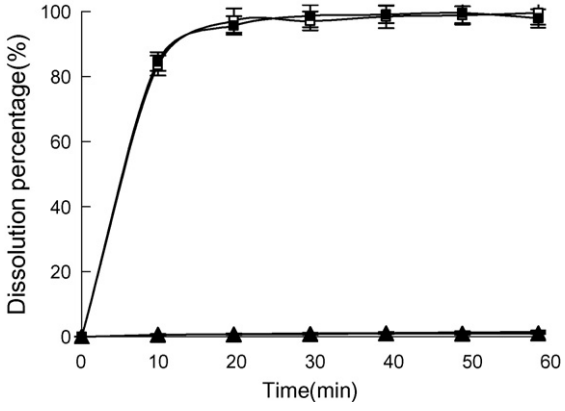


Fig. 6. Dissolution profiles of crude curcumin in pH 1.2 (blank triangle) or in pH 6.8 buffer solution (black triangle) and SMEDDS in pH 1.2 (blank square) or in pH 6.8 buffer solution (black square).

Table 5
The absorption parameters of SMEDDS at different concentrations in rat's intestine *in situ* after perfusing 6 h (mean ± S.D.; $n = 5$).

Dose (mg/kg)	Ka (h^{-1})	$t_{1/2}$ (h)	Uptake percentage (%)
25	0.043 ± 0.004	16.71 ± 1.91	30.11 ± 4.19
50	0.042 ± 0.005	16.31 ± 1.78	29.14 ± 2.89
100	0.043 ± 0.005	15.87 ± 1.81	29.97 ± 3.55

negligible release even after 60 min in both pH 1.2 and 6.8 buffer solutions with the dissolution percentage less than 2%. Whereas, SMEDDS showed rapid dissolution in both solution, at 10 min about 85% of curcumin from SMEDDS was dissolved in medium, and more than 96% was released after 20 min. In other words, SMEDDS could form quickly clear and transparent solution under the condition of dissolution. It was also evident that release of curcumin from SMEDDS was independent of pH dissolution medium.

3.6. *In situ* absorption property of curcumin-loaded SMEDDS in rat intestine

The intestinal absorption kinetics of microemulsification of SMEDDS with low (25 mg/kg), middle (50 mg/kg), high dose (100 mg/kg) was investigated. The results are shown in Table 5. From Table 5, it can be concluded that there was not a significant difference ($p > 0.05$) in absorption parameters such as Ka, $t_{1/2}$ and uptake percentage among variable doses of SMEDDS. From absorption parameters among different doses or concentrations in perfusing solutions (Table 5), curcumin-loaded SMEDDS demonstrated as a concentration-independent absorption in rat's intestine, which might contribute to intestinal absorption of curcumin in SMEDDS *via* passive transfer by diffusion across the lipid membranes.

3.7. *In vivo* oral absorption of curcumin-loaded SMEDDS in mice

There are many metabolites when curcumin is absorbed into blood, some of them are known, and some are unknown, and large part of them exists in blood and a small part can be found in the bile (Zeng et al., 2007; Holder et al., 1978; Shoba et al., 1998). So it is very difficulty to clarify the true absorption of curcumin by determination of curcumin or its metabolites in blood. The oral absorption of curcumin in the study was calculated directly by analyzing the drug amount unabsorbed in gastrointestinal tract and egesta. As shown in Fig. 7, the absorption percentage of curcumin-loaded SMEDDS was higher than that of curcumin suspension at each time point ($p < 0.05$). The absorption percentage of curcumin-loaded SMEDDS (93.8%) at 24 h after administration was 3.86 times that of curcumin suspension (24.3%). The results show that SMEDDS can significantly improve the oral absorption of poorly water-soluble drug.

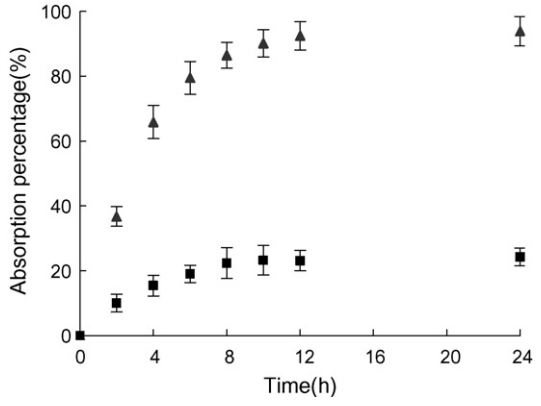


Fig. 7. Absorption percentage of curcumin from SMEDDS (triangle) and suspension (square) at different time after oral administration in mice (mean value ± S.D.; $n = 5$).

4. Discussion

The therapeutic efficiency of curcumin is limited by its poor water solubility and low bioavailability after oral delivery. In this study, a new SMEDDS was applied to improve the solubility and oral absorption of curcumin. When screening a suitable SMEDDS formulation of curcumin, important considerations are included as follows: (1) the selection of simple, safe, and compatible formulation compositions, (2) good solubility of curcumin in various components, (3) a large efficient self-microemulsifying region found in the pseudoternary phase diagram, and (4) the efficient droplet size after forming microemulsion (Zhang et al., 2008). Therefore, the selection of oil, surfactant and co-surfactant as well as the mixing ratio of oil to surfactant/co-surfactant plays an important role in SMEDDS formulation. Based on the solubility studies of curcumin in several tested vehicles and the mutual compatibility tests, the mixtures of emulsifier OP:EL-40 (1:1, w/w), PEG 400 and ethyl oleate were selected as surfactant, co-surfactant and oil, respectively.

A simplex lattice experiment design was adopted to optimize the system compositions in which many factors were evaluated by changing their concentrations simultaneously and keeping their total concentration constant. The solubility and mean particle size were the key parameters for SMEDDS (Holm et al., 2006). Therefore, they were chosen as the responses to screen the three variables such as the contents of surfactant, co-surfactant and oil of SMEDDS. From the regression Eqs. (2) and (3) obtained by Matlab Software, it is concluded that the contents of three ingredients or the interactions among them can have significant effects on the particle size of microemulsion formed from SMEDDS and the solubility of curcumin in SMEDDS at the chosen content scopes, which was in accordance with the results reported by Holm et al. (2006).

As given in Table 4, minor difference in the droplet size was observed by varying the diluents (deionized water, 0.1 N HCl, and pH 6.8 phosphate buffer). It indicated that the resulting formulations were not significantly affected by pH and the ionic strength. Electrical conductivity and mean particle size were decreased noticeably after loading curcumin. The possible reason is that there are two aldehyde groups and two hydroxy groups in curcumin molecule structure as shown in Fig. 1, when curcumin molecule is dissolved and dispersed into the emulsifying membrane layer (composed of surfactant and co-surfactant) and oil phase, these groups in curcumin molecule can react with ethyl oleate, emulsifier OP, Cremorphor EL and PEG 400, producing a lot of hydrogen bonds. Thus the whole particle shrinks due to the interaction of above hydrogen bonds and consequently, the mean particle size of resultant microemulsion decreases. Because of the formation of hydrogen bonds among curcumin molecule, emulsifier OP, Cremorphor EL and PEG 400, the number of hydrophilic groups exposed to water in emulsifying membrane layer diminishes. As a result, the electrical conductivity of microemulsion solution decreases as well.

In order to investigate transport mechanism of curcumin in SMEDDS, the method of perfusion in rat intestine was used in the present study because it can give a reliable prediction of oral absorption of drug in humans (Zakeri-Milania et al., 2007). In rat intestinal perfusing experiment, a dye (e.g., phenol red) was added into the sample solution as a non-absorbed marker. However, phenol red was reported to be partly absorbed in small intestine and may interfere with the transport of some definite drugs, especially the poorly water-soluble drugs (Hu et al., 1996). In order to overcome the disadvantages, many simply methods such as gravimetric determination and direct-volume recording were adopted (Liao et

al., 2005; Ritesh et al., 2007). In this paper, the residue drug was calculated by recording volume of perfusion solution in circulation system directly at predetermined intervals. Our investigation showed the error between volume values by recording directly and real volumes of perfusion solution at predetermined intervals was less than 3%.

As shown in the Fig. 7, the oral absorption of curcumin, a poorly water-soluble drug, was enhanced by SMEDDS. There are several possible reasons. When curcumin-loaded SMEDDS is administrated to the gastrointestinal tract, the spontaneous formation of a microemulsion presenting curcumin in a dissolved form will be beneficial to enhance its absorption. In addition, as compositions in SMEDDS, surfactants can reduce the interfacial surface tension and enhance penetration of curcumin to the epithelial cells. Furthermore, there are a lot of lymphatic tissues such as Peyer's patches and Microfold cells in rat intestine, because the microemulsion droplets produced from SMEDDS in the perfusion can be uptaken *via* the lymphatic tissues (Narang et al., 2007), the absorption *via* these lymphatic tissues could be an important route for curcumin as well.

5. Conclusion

A SMEDDS formulation for curcumin was developed and optimized with a simplex lattice method, and the optimal formulation was as follows: 57.5% surfactant (28.75% emulsifier OP and 28.75% Cremorphor EL), 30% co-surfactant (PEG 400) and 12.5% oil (ethyl oleate). The solubility of curcumin in SMEDDS was 21 mg/g. When diluted with water, curcumin-loaded SMEDDS could spontaneously form small particles with average particle size of about 21 nm. Dissolution percentage of curcumin in SMEDDS in pH 1.2 or 6.8 buffer solution was significantly higher than that of crude curcumin. The absorption of curcumin-loaded SMEDDS in rat intestine was *via* passive transfer by diffusion across the lipid membranes. The oral absorption of curcumin *in vivo* in mice was significantly enhanced by SMEDDS compared with curcumin suspension. Our studies show that SMEDDS is a promising formulation for improving oral absorption of drug with poor aqueous solubility.

Acknowledgement

This work is supported by a research grant (2006GG2202053) from Department of Shandong Science and Technology, PR China.

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