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ORIGINAL ARTICLE

Self-microemulsifying drug delivery system improves curcumin dissolution and bioavailability

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Abstract

Background: Curcumin has a wide spectrum of biological and pharmacological activities, but it has not yet been approved as a therapeutic agent because of its low solubility and stability in aqueous solution, and the relatively low bioavailability in vivo. To overcome these limitations, self-microemulsifying drug delivery system (SMEDDS) of curcumin was developed. *Method*: Various oils, surfactants, and cosurfactants were selected to optimize the formulation. Pseudoternary phase diagrams were constructed and orthogonal design was used to compare the oil-in-water (o/w) microemulsion-forming capacity of different oils/surfactants/cosurfactants. The solubility of curcumin in various oils and cosurfactants was determined to find suitable ingredients with a good solubilizing capacity. Droplet size was measured to obtain the concentration of oil, surfactant, and cosurfactant for forming stable microemulsion. Furthermore, its quality and bioavailability in mice were assessed. *Results:* Pseudoternary phase diagrams and solubility test showed that the formulation of SMEDDS composed of 20% ethanol, 60% Cremophor RH40®, and 20% isopropyl myristate, in which the concentration of curcumin reached 50 mg/mL. Curcumin was released completely from SMEDDS at 10 minutes. The developed SMEDDS formulation improved the oral bioavailability of curcumin significantly, and the relative oral bioavailability of SMEDDS compared with curcumin suspension was 1213%. *Conclusion:* The SMEDDS can significantly increase curcumin dissolution in vitro and bioavailability in vivo.

Key words: Bioavailability, curcumin, dissolution, SMEDDS, stability

Introduction

Curcumin, a hydrophobic polyphenol derived from the rhizome of the herb *Curcuma longa*, has a wide spectrum of biological and pharmacological activities, including antioxidative, anti-inflammatory[1–3], antimicrobial, and anticarcinogenic activities[4–6]. Various animal models[7,8] or human studies[9–11] proved that curcumin is extremely safe even at very high doses. For example, three different phase I clinical trials indicated that curcumin, when taken as high as 12 g/day, is well tolerated[9,10]. Similarly, the efficacy of curcumin in various diseases including cancer has been well established[12]. The pharmacological safety and efficacy of curcumin makes it a potential compound for treatment and prevention of a wide variety of human diseases. Despite its efficacy and safety, curcumin has not yet been approved as a therapeutic agent; its low solubility[13] and stability[14] in aqueous solution and the relatively low bioavailability of curcumin[15] have been highlighted as two major problems for this. These limitations should be overcome to enhance the bioavailability by using novel drug delivery systems.

One of the most appropriate breakthroughs toward the problems is the use of self-microemulsifying drug delivery system (SMEDDS) that consist of a mixture of drug, oil, surfactant, and cosurfactant, and the gentle mixing of these ingredients in aqueous media can generate oil-in-water (o/w) microemulsion droplets of solubilized drugs with a mean droplet size ≤100 nm[16]. It is considered that such SMEDDS may protect labile drug, control drug release, increase drug solubility, improve the absorption of drugs by rapid self-microemulsification in the stomach, with the microemulsion droplets

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subsequently dispersing in the gastrointestinal tract, thereby allowing them to be readily absorbed[17–19]. Furthermore, it has proven possible to formulate preparations suitable for most routes of administration[20,21].

There are several studies on SMEDDS or microemulsion as carrier of curcumin, and it has been shown that such formulations have improved transdermal delivery properties[22] and oral absorption in situ[23]. But much needs to be done to prove that SMEDDS as a carrier of curcumin can increase its bioavailability in vivo. The objectives of our study were to design, prepare, and characterize curcumin SMEDDS, and assess its bioavailability.

In this study, to develop a SMEDDS containing poorly water-soluble curcumin, various oils, surfactants, and cosurfactants were selected to optimize the formulation. Pseudoternary phase diagrams were constructed and orthogonal design was used to compare the o/w microemulsion-forming capacity of different oils/surfactants/cosurfactants. The solubility of curcumin in various oils and cosurfactants was investigated to find suitable ingredients with a good solubilizing capacity. Furthermore, droplet size was determined to obtain the concentration range of oil, surfactant, and cosurfactant for curcumin SMEDDS. Its quality in vitro and its bioavailability in mice in vivo were assessed.

Table 1. The factors and levels of the orthogonal test.

	Factors		
Levels	Oil (A)	Surfactant (B)	Cosurfactant (C)
1	Isopropyl myristate	Tween 80	Ethanol
2	Aethylis oleas	Cremophor RH40	PEG 400
3	Soybean oil	Cremophor EL	1,2-propylene glycol

Table 2. The results of the orthogonal test.

	Factors			
Experiment num.	A	B	C	Plot num.
1	1	1	1	51
2	1	2	2	43
3	1	3	3	72
4	2	1	3	47
5	2	2	1	81
6	2	3	2	41
7	3	1	2	3
8	3	2	3	10
9	3	3	1	0
X_1	166	101	132	
X_2	169	134	87	
X_3	13	113	129	
$\overline{X}_1$	55.33	33.67	44	
$\overline{X}_2$	56.33	44.67	29	
$\overline{X}_3$	4.33	37.67	43	
R	52	11	15	

Materials and methods

Materials

Curcumin was purchased from Shanghai Reagent Co., Ltd. (Shanghai, China). Cremophor EL® (polyoxyl 35 castor oil) and Cremophor RH40® were purchased from BASF (Ludwigshafen, Germany). Isopropyl myristate (IPM), aethylis oleas, ethanol, 1,2-propylene glycol, PEG 400, and Tween 80 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Soybean oil was provided by Tieling Beiya Pharmaceutical Oil Co. Ltd. (Liaoning, China). High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). All the other chemicals used were of analytical reagent grade.

Phase studies

Various oils, surfactants, and cosurfactants were selected to formulate SMEDDS containing curcumin. Orthogonal design $L_9(3^4)$ in three levels of three factors (oil, surfactant, and cosurfactant, as shown in Table 1) was used to screen the proper ingredients with high o/w microemulsion-forming capacity. $L_9(3^4)$ orthogonal design for the factors and levels is shown in Table 2. Pseudoternary phase diagram was constructed by using a conventional titration technique. Briefly, appropriate amounts of oil, surfactant, and cosurfactant were taken in different stoppered test tubes and vortexed vigorously to ensure thorough mixing. These mixtures were then dropped into 100 mL of water at 37°C and stirred gently at constant 100 rpm to reach equilibrium. Transparent, low-viscous single phases were identified as microemulsion. The efficient self-microemulsification domain was plotted on a pseudoternary phase diagram with one axis representing oil, one representing surfactant, and the third representing cosurfactant. The existence area of self-microemulsification domain was represented by the plotted numbers that can form microemulsion, and the statistical analysis was performed. The components with larger self-microemulsification domain were selected to perform solubility test.

Solubility of curcumin

Suitable oil and cosurfactant that possess good solubilizing capacity on curcumin were identified by using solubility studies. Briefly, excessive curcumin (100 mg) was added into 2 g of various oils or cosurfactants and shaken at 37°C (420 constant temperature shaker, Forma Scientific Marietta, Ohio, USA) for at least 48 hours. The suspensions were centrifuged at 1500 × g for 10 minutes (Anting centrifuge TDL-50B, Shanghai Anting Scientific Instrument Factory, Shanghai, China) to remove the undissolved drugs. Aliquots of supernatant were taken and appropriately diluted with acetonitrile. Drug concentration in the supernatant was analyzed by HPLC

(Shimadzu LC-20AB, Shimadzu Corporation, Kyoto, Japan)[24].

The effects of drug on the phase diagram

The following experiment was carried out to investigate the effects of curcumin on the self-emulsifying performance of SMEDDS. The formulation amount of curcumin was added to the formulations of the self-emulsifying domain of the ternary phase diagrams. The self-emulsifying performance was visually assessed after infinite dilution using purified water.

Effects of oil and surfactant/cosurfactant ratio on droplets dimensional distribution

SMEDDS were prepared with the oil amount varied 10–50% and surfactant/cosurfactant at different mass ratios (7:1, 6:2, 5:3, 4:4, 3:5). These SMEDDS were diluted with purified water to obtain microemulsion and the particle size distribution was determined immediately using NICOMP 380 Particle/Zeta Sizer (Santa Barbara, CA, USA), which is based on the principle of photon correlation spectroscopy. The particle sizing system covers the size from 5 nm to 3 μm and performs at a fixed angle of 90°.

Characterization of curcumin SMEDDS

Microemulsion systems were characterized in terms of pH, surface charge, droplets dimensional distribution, and images of transmission electron microscope. The pH values of the samples were measured by a pH meter (model PHS-2C, Lida equipment mill, Shanghai, China) at (20 + 2°C). The droplets dimensional distribution and zeta potential were measured at room temperature using NICOMP 380 Particle/Zeta Sizer. Transmission electron microscopy (TEM, H-7000, Hitachi Ltd., Tokyo, Japan) was employed to characterize the microstructure of curcumin microemulsion with the method reported by Chen et al.[25]

Determination of emulsification time

The emulsification time of SMEDDS was determined according to Chinese Pharmacopoeia (2005 version)[26], dissolution apparatus 3 (D-800LS intellectualized dissolution apparatus, Tianda Tianfa-Pharmaceutical Testing Instrument Manufacturer, Tianjin, China). One gram of each formulation was added into 100 mL of distilled water, pH 6.8 phosphate buffer saline (PBS) and 0.1N HCl at 37°C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 100 rpm. Three milliliter sample was collected at 1, 2, 3, 4, 5, 6, 8, 10 minutes, and the turbidity was measured at 550 nm. The time of self-emulsification was recorded by turbidity that became unchanged when self-emulsification reached equilibrium[27].

Dissolution

Dissolution studies were performed as follows: SEMDDS containing 10 mg of curcumin was introduced into 900 mL dissolution medium consisting of 0.25% sodium dodecyl sulfate (SDS) in double-distilled water at 37 ± 0.5°C. The revolution speed of the paddle was maintained at a rate of 100 rpm. An aliquot (0.5 mL) of sample was collected at designated times, centrifuged for 5 minutes, and supernatant was analyzed for curcumin by HPLC. An equivalent volume (0.5 mL) of fresh dissolution medium was added to compensate for the loss because of sampling. The release of curcumin from SMEDDS formulation was compared with curcumin crude powder containing the same quantity of drug.

Stability

The stability was assessed by analyzing appearance, droplet size, and content at 0, 1, 2, 4, and 8 hours after SMEDDS formulation was dispersed in distilled water, 0.1N HCl, pH 6.8 PBS. The curcumin SMEDDS formulations were tightly sealed in vials for storage at ambient temperature (25°C) for 1 month and refrigerated (4°C) for 7 days and the content of curcumin and appearance were evaluated.

Bioavailability

The in vivo study was carried out with male mice (18–22 g) supplied by Medical Animal Test Center of Fujian Medical University. All procedures of laboratory animals involved were in accordance with National Institutes of Health guidelines. Mice were randomly divided into two groups: curcumin microemulsion (group A) which was prepared by diluting the curcumin SMEDDS with distilled water and curcumin suspension (group B), and each group consisted of five mice. Curcumin was given orally or gavage at the same dose of 200 mg/kg. Curcumin suspensions were prepared by suspending the curcumin into 0.5% sodium carboxymethylcellulose solution. Prior to the experiment, the animals were fasted overnight but had free access to water. Mice were sacrificed at various time points after administration, and blood samples (~600 μL) were collected into a heparinized microcentrifuge tube from the retro-orbital vein. Samples were then subjected to centrifugation for 5 minutes at 4°C (7000 × *g*) and plasma was withdrawn and stored at −20°C until analysis.

Curcumin concentration in blood was determined by reversed-phase HPLC. Briefly, exactly 200 μL of the plasma sample was mixed with 500 μL of ethyl acetate, and vortexed for 3 minutes using a vortex mixer (MS 2 IKA Minishaker, IKA® Werke GmbH & Co. KG, Staufen, Germany). The mixture was then centrifuged at 630 × *g* for 5 minutes using a high-speed centrifuging machine (Anting TGL-20C high-speed centrifuging machine, Shanghai Anting Scientific Instrument Factory, Shanghai, China). The supernatant was taken and another 500 μL of ethyl acetate was added into the precipitation for the second extraction. The supernatants of two times extraction were combined and dried with N_2. The residue was resolved with 100 μL mobile phase

consisted of acetonitrile, water, and glacial acetic acid (50:47.5:2.5, volume ratio). After centrifugation at 10,000 rpm for 5 minutes, 25 μL of supernatant was injected into the HPLC system. Sample detection was achieved at 428 nm. The column temperature was set to be 30°C with a flow rate of 1.0 mL/min.

The mean recovery was above 95.8% with a coefficient of variation below 7%. The linear regression equation of the calibration graph of curcumin was $A = 296.54C + 1853.1$ ($r = 0.9940$). The linear range for the determination of curcumin was 21–420 ng/mL, and the limit of detection ($S/N > 3$) was 6.3 ng/mL. At concentrations of 31.5, 168, and 378 ng/mL, extraction recoveries of curcumin from mouse plasma were 86.34%, 89.49%, and 80.85%, respectively; intraday precision was 7.88%, 4.07%, and 5.24%; and interday precision was 4.25%, 3.77%, and 3.19%.

The pharmacokinetic parameters were evaluated by software DAS 2.0, a computer program produced by Drug Research Center of Shanghai University of Traditional Chinese Medicine. The AUC_{0-t} was the area under the plasma concentration–time curve from time 0 to final observed concentration time point upon oral administration that was calculated using the linear trapezoidal rule. The $AUC_{t-\infty}$ is the terminal area under the plasma concentration–time curve from time t to infinity, calculated by dividing the last observed plasma concentration by λz, where λz denotes the first-order rate constant of the terminal phase. The maximum serum concentration (C_{max}) and the time to reach the peak serum concentration (T_{max}) were obtained directly from the experimental data. Relative bioavailability can be calculated according to Equation (1):

$$\text{Relative bioavailability} = \frac{AUC_T}{AUC_R} \times 100\%. \quad (1)$$

Results and discussion

Phase studies

The consideration for screening formulation of SMEDDS usually involves the following: the formulation composition should be simple, safe, and compatible; a large efficient self-microemulsification region should be found in the pseudoternary phase diagram; it should possess good solubility and have efficient droplet size after forming microemulsion[28–31].

Hydrophilic–lipophilic balance (HLB) refers to the relative attraction of a surfactant or emulsifier for water and oil. The efficiency of self-microemulsification is much related to the HLB of the surfactant. Generally, surfactants with HLB 12–15 are regarded as being of good efficiency. Considering the safety and biocompatibility of the excipient, we selected three kinds of nonionic surfactants, namely, Cremophor EL (HLB 12–14), Cremophor RH40 (HLB 12–14), and Tween 80 (HLB 15), combined with ethanol, propylene glycol, or PEG 400 as cosurfactant which can adjust the HLB of surfactant, reduce the interfacial tension, and be beneficial to form fine droplets and hence increase the stability of microemulsion. Moreover, cosurfactant can increase the solubility of hydrophobic drug[32].

$L_9(3^4)$ orthogonal experiment was designed to compare the efficient self-microemulsification capacity of different oils, surfactants, and cosurfactants. Pseudoternary phase diagrams were used to identify the area of microemulsion regions, which is shown in Figure 1. The base design consisted of nine runs. The ninth run cannot form transparent microemulsion, so the pseudoternary phase diagram was not drawn. The existence area of self-microemulsification domain was represented by the plotted numbers that can form microemulsion. $\bar{X}$ represents the self-microemulsification capacity of the corresponding factor, the larger the $\bar{X}$, the greater the self-microemulsification capacity. As listed in Table 2, $\bar{X}$ of IPM, aethylis oleas, and soybean oil are 56.33, 55.33, and 4.33, respectively, which demonstrate that the self-microemulsification capacity of IPM and aethylis oleas are superior to soybean oil. Therefore, IPM and aethylis oleas were chosen to perform the solubility study. Similarly, Cremophor RH40 has the best self-microemulsification capacity among three types of surfactants. Ethanol and 1,2-propylene glycol were selected as the cosurfactants of SMEDDS because they have approximate $\bar{X}$ and superior to PEG 400.

Solubility of curcumin

Appropriate vehicles should have good solubilizing capacity of the drug substance, which is essential for allowing presentation of the drug in solution. The results of solubility of curcumin in the vehicles which have greater self-microemulsification capacity are shown in Table 3. Ethanol provided higher solubility than 1,2-propylene glycol, so was chosen as the cosurfactant for the optimal SMEDDS formulation. Solubility of curcumin in IPM and aethylis oleas at 37°C was approximate, but aethylis oleas could not be used because the solubility in this solvent decreased apparently with the appearance turning from transparent to opaque when temperature dropped to ambient temperature. Therefore, IPM was selected as oil of curcumin SMEDDS. Solubility of curcumin in Cremophor RH40 was measured by adding drug into solvent gradually because Cremophor RH40 is very viscous. Cremophor RH40 has good solubilizing capacity of curcumin with the solubility being more than 150 mg/g.

Effects of drug on the phase diagram

It has been reported that the drug incorporated into the SMEDDS may have some effect on the self-emulsifying performance[33], so the effect of drug loading on the area of self-emulsion region of phase diagram was studied,

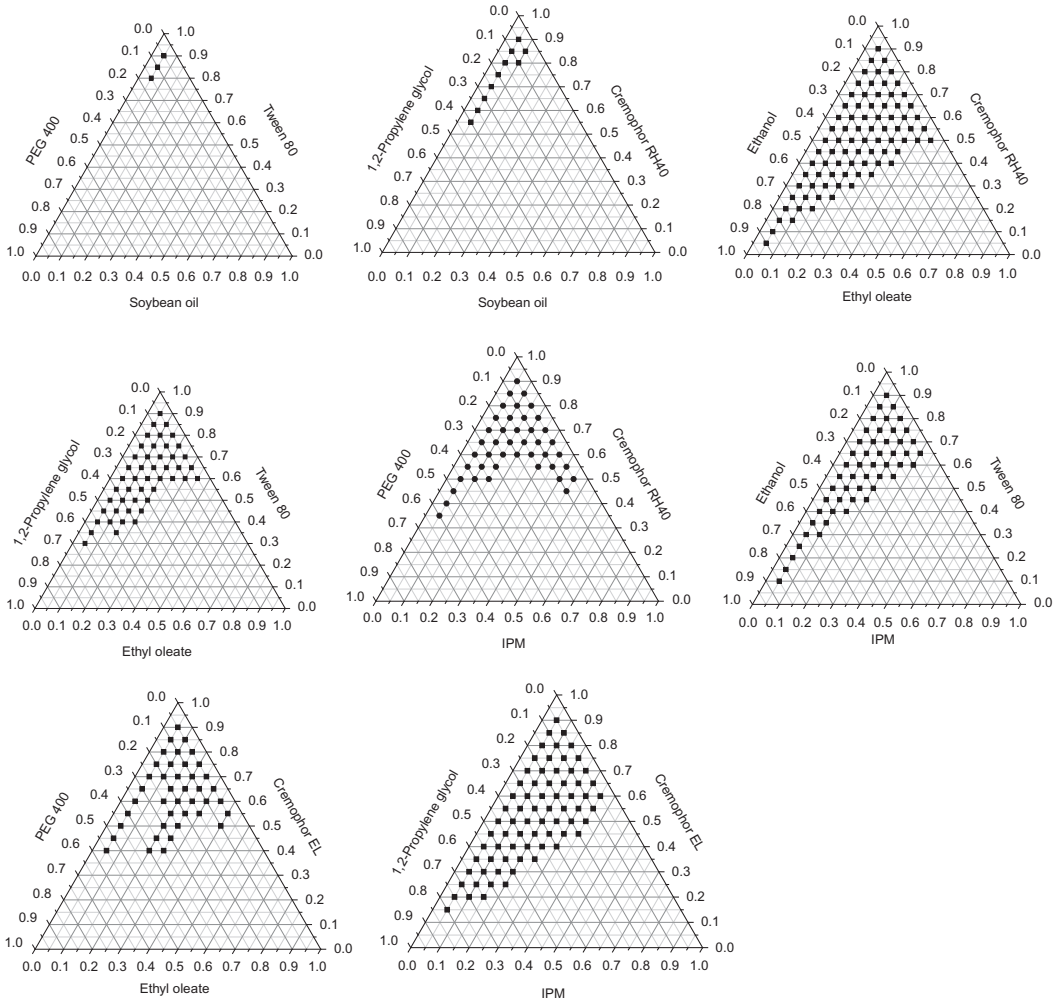

Figure 1. Pseudoternary phase diagrams for formulas ('■', efficient self-emulsification region).

Table 3. Solubility of curcumin in different excipients ($n = 3$).

Excipients	Solubility (μg/g)
Isopropyl myristate	1760.93 ± 99.84
Ethyl oleate	1710.43 ± 75.05
1,2-Propylene glycol	7830.07 ± 1120.81
Ethanol	27,122.54 ± 1905.38
Cremophor RH40	>150,000

which is shown in Figure 2. The efficient self-microemulsion region was smaller than blank SMEDDS, so it is reasonable to conclude that the self-microemulsion capacity was affected by curcumin. The reasons may include the following: (1) some of the formulations cannot solubilize curcumin completely which makes the system turbid before and after self-microemulsification in water; (2) curcumin existing in the surfactant may interfere with its capacity to decrease the surface tension and hinder cosurfactant intercalation into the moecules of surfactant to form surface membrane, hence influencing the efficient self-microemulsion region.

Effects of oil and surfactant/cosurfactant ratio on droplets dimensional distribution

Particle size after microemulsification was the most important property of SMEDDS. Mechanisms of particle size effect on drug absorption may include improved release and facilitated lymphatic transport[34–37].

The effect of the concentration of oil on the droplet size was investigated when the surfactant/cosurfactant ratio was fixed as 9:1. SMEDDS formulation was dispersed into purified water. The droplet size increased from 19.9 to 107.3 nm (Gaussian distribution) when the concentration of oil added increased from 10% to 50%. When the concentration of oil was more than 30%, double peaks appeared in the droplet size distribution (Nicomp distribution) which demonstrated that the system became unstable. So it is reasonable to fix the oil percentage at 20% (Table 4).

The ratio of surfactant to cosurfactant was very critical for forming a stable and efficient SMEDDS formation. Therefore, the influence of the different ratios of surfactant (Cremophor RH40) to cosurfactant (ethanol) on the droplet size was also investigated. Under the condition

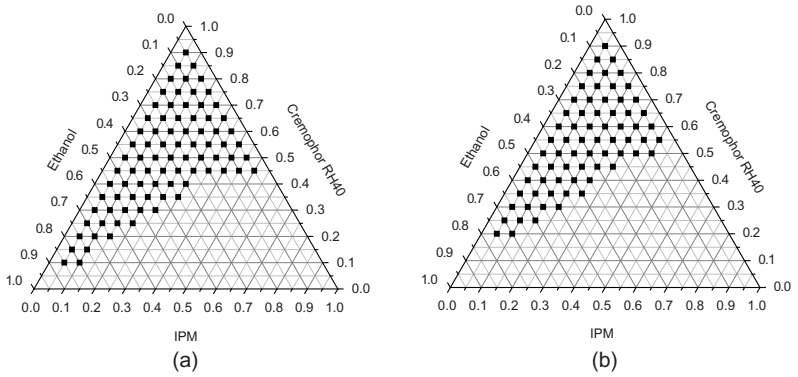

Figure 2. Pseudoternary phase diagrams of (a) not including curcumin and (b) including curcumin.

Table 4. Effect of different oil ratios on mean droplet size (nm, $\bar{X} \pm$SD).

		Nicomp distribution			
Oil ratio (%)	Gaussian distribution	Peak 1	(%)	Peak 2	(%)
10	19.9 ± 8.60	19.5 ± 2.5	100	—	—
20	29.4 ± 15.9	19.5 ± 2.5	100	—	—
30	34.8 ± 14.6	23.5 ± 2.9	44.68	55.7 ± 7.0	55.32
40	68.2 ± 26.7	17.7 ± 1.9	2.94	68.6 ± 9.0	97.06
50	107.3 ± 55.3	28.1 ± 2.9	6.11	115.3 ± 15.5	93.89

of fixed oil proportion at 20%, the droplet size increased (Gaussian distribution) when the ratio of surfactant to cosurfactant decreased from 7:1 to 3:5. Moreover, double peaks appeared when the ratios of surfactant to cosurfactant were at 5:3, 4:4, and 3:5 (Nicomp distribution), which demonstrated that the system became unstable eventually when cosurfactant increased. Although nonionic surfactants such as Cremophor Rh40 are less toxic than ionic surfactants, they may lead to reversible changes in the permeability of the intestinal lumen. It is very important to determine the surfactant concentration properly as large amounts of surfactants may cause gastrointestinal irritation[38]. Usually, the surfactant concentration ranges between 30% and 60% (w/w). In addition, lowering the surfactant content can decrease the viscosity of the relevant formulation, hence improve the emulsification rate[39]. So the optimal ratio of surfactant to cosurfactant was selected to be 6:2 (Table 5).

Based on the results, a three-component SMEDDS formulation was established: 20% IPM as oil, 60% Cremophor RH40 as surfactant, and 20% ethanol as cosurfactant. Curcumin amount was 50 mg/g. This optimized curcumin SMEDDS was used for further experiments.

Determination of emulsification time

Self-microemulsification efficiency refers to the capacity of SMEDDS to spontaneously form or disperse into microemulsion with fine and homogeneous particles when subjected to aqueous dilution under mild agitation. The rate or time of self-emulsification was recorded to evaluate the self-microemulsification efficiency[26]. The time of self-emulsification in water, 0.1M HCl, and pH 6.8 PBS were 3, 4, and 1 minutes, respectively, which suggested that self-microemulsification efficiency of curcumin SMEDDS in intestinal tract was better than in stomach.

Dissolution

Release of curcumin crude drug powder and SMEDDS was compared (Figure 3). Curcumin was released completely from SMEDDS at 10 minutes, whereas the release percentage of curcumin crude drug powder was limited to 41.22% within 60 minutes because of its hydrophobic property. Statistically significant difference was observed between the two kinds of curcumin forms ($P < 0.05$). It could suggest that curcumin dissolved perfectly in SMEDDS

Table 5. Effect of surfactant to cosurfactant ratio on mean droplet size (nm, $\bar{X} \pm$SD).

		Nicomp distribution			
Ratio (surfactant/cosurfactant)	Gaussian distribution	Peak 1	%	Peak 2	%
7:1	29.3 ± 8.2	26.8 ± 2.8	100	—	—
6:2	27.3 ± 11.1	25.8 ± 3.5	100	—	—
5:3	58.3 ± 23.3	11.3 ± 1.0	3.47	55.2 ± 4.1	96.53
4:4	113.8 ± 53.0	48.8 ± 6.5	20.66	157.4 ± 21.5	79.34
3:5	105.4 ± 57.4	48.0 ± 5.3	23.67	168.4 ± 18.1	76.33

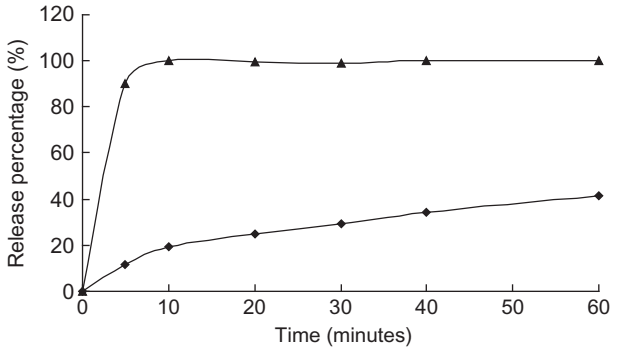

Figure 3. Release percentage of curcumin SMEDDS and curcumin powder (▲, curcumin SMEDDS; ◆, curcumin powder).

form could be released because of the small droplet size, which permits a faster rate of drug release into aqueous phase, and it could affect the bioavailability.

Characterization of curcumin SMEDDS

Following 1:10 aqueous dilution of the optimal curcumin SMEDDS formulation, the droplet size of the microemulsion was 32.9±19.3 nm for intensity weighting Gaussian distribution, and 27.8±3.2 for intensity weighting Nicomp distribution, as shown in Figure 4. The resulting microemulsions were approximate to neutral. The pH value of the microemulsions was around 6.3 and zeta potential was −19.69 mv. The TEM picture suggested that the microemulsion particles were almost of spherical shape with smooth surface. The appearance was transparent with visible orange opalescence. The average droplet size of microemulsion dispersed was within 100 nm and was consistent with the data analyzed using particle sizing apparatus.

Stability

There were no obvious changes with the appearance and content when curcumin SMEDDS was dispersed in three kinds of medium after standing for 8 hours. The mean droplet size was increased eventually but was not more than 110 nm within 8 hours. There was no major change in the content and appearance of curcumin SMEDDS when stored at 25°C and 4°C within the designated time.

Bioavailability

The plasma profiles of curcumin in mice following gavage of curcumin suspension or microemulsion were compared. Figure 5 shows that plasma concentration profile of microemulsion was significantly different from that of the curcumin suspension. Pharmacokinetic parameters are shown in Table 6.

After gavage administration of suspension, plasma level of curcumin was very low, most of which (after 0.5 hour) was under limit of detection, with $AUC_{0-\infty}$ and C_{max} of only 21.76 μg·h/L and 63.89 μg/L. As for microemulsion, plasma level of curcumin can be detected even after 4 hours, and the $AUC_{0-\infty}$ and C_{max} value were 277.06 μg·h/L and 196.56 μg/L. Relative bioavailability of microemulsion was dramatically enhanced compared to curcumin suspension. The bioavailability of microemulsion was 12.73-fold that of curcumin suspension. Therefore, it is reasonable to conclude that microemulsion enhances the absorption and retention time in vivo significantly.

There are many reports about the bioavailability of curcumin. The reasons for poor bioavailability of this agent within the body include the following: (1) poor absorption which is due to the low solubility of curcumin in water or in gastrointestinal tract. The first reported study to examine course of curcumin in vivo was by Wahlstrom and Blennow[40]. Negligible amounts of curcumin in blood plasma of rats after oral administration of 1 g/kg of curcumin showed that curcumin was poorly absorbed from the gut. (2) High rate of metabolism. Once absorbed, curcumin is subjected to conjugations like sulfation and glucuronidation at various tissue sites. Hydrolysis of plasma samples with glucuronidase showed that 99% of curcumin in plasma was present as glucuronide conjugates[41]. (3) Systemic elimination or clearance of curcumin from the body is also an important factor, which determines its relative biological activity. The elimination half-life of orally administered

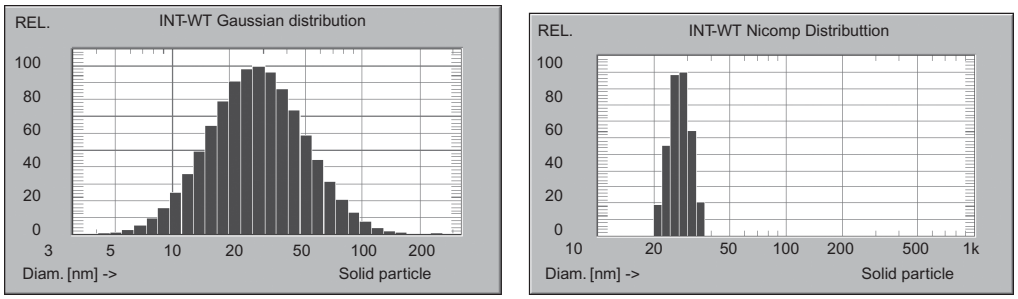

Figure 4. The particle diameter of curcumin SMEDDS dispersions. (a) Intensity weighting Gaussian distribution; (b) intensity weighting Nicomp distribution.

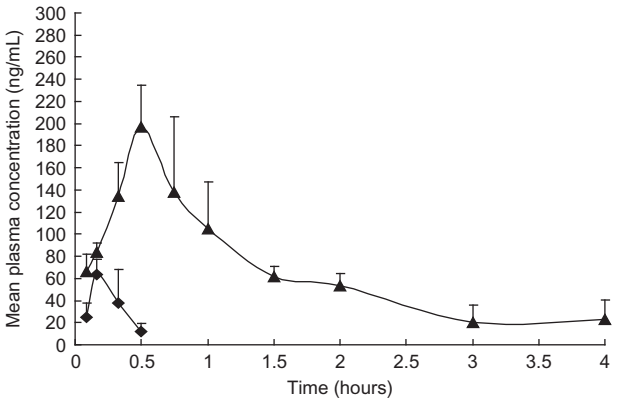

Figure 5. The profiles of mean drug plasma concentration–time after the gavage administration of single curcumin suspensions and microemulsion (▲, curcumin microemulsion; ◆, curcumin suspension).

Table 6. Main pharmacokinetics parameters of curcumin microemulsion and suspension.

Parameters	Unit	Microemulsion	Suspension
$t_{1/2}$	hours	0.74	0.092
Ke	1/h	0.94	7.513
$AUC_{0-\infty}$	μg/L·h	277.06	21.76
K_a	1/h	3.91	11.34
T_{max}	hours	0.5	0.17
C_{max}	μg/L	196.56	63.89

curcumin (2 g/kg) in rats was reported to be 1.7 ± 0.5 hours[42].

Similar low bioavailability was obtained in the present study about curcumin suspension, although curcumin SMEDDS can overcome this drawback to some extent. First of all, SMEDDS improves the solubility of lipophilic drugs in aqueous circumstance which is the prior condition of absorption, and the small droplet size in microemulsion provides a large interfacial surface area for the improved absorption[43]. Second, the presence of surfactant in SMEDDS might have caused changes in the permeability by disturbing the cell membrane[25]. Surfactant monomers are capable of partitioning into the cell membrane where they can form polar defects in the lipid bilayer. At the same time, surfactant also demonstrated a reversible effect on the opening of tight junction; it may interact with the polar head groups of the lipid bilayers, modifying hydrogen bonding and ionic forces between these groups[44]. Moreover, it was reported that the long-chain oils promote lipoprotein synthesis and subsequent lymphatic absorption[45].

Cui et al.[23] investigated the oral absorption of curcumin SMEDDS in mice. The authors revealed that the absorption percentage of curcumin-loaded SMEDDS at 24 hours after administration was 3.86 times that of curcumin suspension. From the results of our study, the bioavailability of SMEDDS was 12.73-fold that of curcumin suspension. So besides the increase of absorption, it must involve other mechanisms to improve the bioavailability. Phase I metabolism by the intestinal cytochrome P450s is now becoming recognized as a significant factor in oral drug bioavailability. In an in vitro study, when everted sacs of rat intestine were incubated with 5–75 mg/L of curcumin, 30–80% of the curcumin disappeared from the mucosal side and no curcumin was found in the serosal fluid, which means degradation takes place during the absorption process[15]. SMEDDS, a lipid-based formulation, is considered to be partially absorbed via the lymphatic route. That may reduce the opportunity for pre-systemic drug metabolism and hepatic first pass metabolism, therefore enhancing the bioavailability of drugs[46]. Constantinides[29] also revealed that the drug compound in o/w microemulsions reaches the capillaries incorporated within the oil droplets, which can protect drug from chemical as well as enzymatic degradation, increase the residence time, and improve the bioavailability in vivo. In our study, the $t_{1/2}$ of microemulsion was 0.74 hours which is about eight times of that of suspension, which means microemulsion decreased the rate of elimination of curcumin. Therefore, the increase of bioavailability offered by SMEDDS may contribute not only to the absorption but also to the degradation process in vivo.

Conclusions

SMEDDS formulations containing curcumin were prepared and the quality and bioavailability were evaluated in vitro and in vivo. Through pseudoternary phase diagrams and orthogonal experiment of $L_9(3^4)$, the o/w microemulsion-forming capacity of different oils/surfactants/cosurfactants were compared. The solubility of curcumin in various oils and cosurfactants was investigated to find suitable ingredients with a good solubilizing capacity. Furthermore, droplet size was determined to obtain the concentration range of oil, surfactant, and cosurfactant for curcumin SMEDDS. The pH, self-emulsification time, particle size, stability, and dissolution of the resultant emulsion after self-emulsification were determined. The plasma concentrations in mouse were determined by HPLC and the pharmacokinetics behavior of curcumin microemulsion was evaluated by comparison with suspension. The curcumin self-microemulsifying delivery system can be emulsified completely within 4 minutes. Mean particle size of the resultant emulsion was 31.98 nm, and pH approximated to neutral. The dissolution of curcumin self-microemulsifying formulation at 10 minutes was 100% and the content of drug maintained above 94% with 8 hours. Compared with suspension, AUC of microemulsion after gavage increased 12-fold. The self-microemulsifying delivery system can significantly increase curcumin dissolution in vitro and bioavailability in vivo.

Declaration of interest

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