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Preparation and Oral Bioavailability Study of Curcuminoid-Loaded Microemulsion

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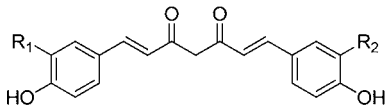
ABSTRACT: Curcuminoid, a dietary polyphenolic compound, has poor water solubility and low bioavailability following oral administration. The aim of this study was to develop a formulation of curcuminoid-loaded microemulsion (Cur-ME) to improve its oral bioavailability. The optimized Cur-ME formulation was prepared by using labrafac lipophile WL 1349, cremophor RH 40, and glycerine as the oil phase, the surfactant, and the cosurfactant, respectively. Pharmacokinetics and bioavailability of curcuminoid suspension and Cur-ME were evaluated and compared in rats. Plasma bisdemethoxycurcumin (BDMC), treated as the representing component of curcuminoid, was determined by high-performance liquid chromatography with fluorescence detector. After gavage administration of curcuminoid suspension, the plasma BDMC level was very low, below 5 ng/mL, whereas for Cur-ME, double peak of maximum concentrations were observed. The relative bioavailability of Cur-ME was enhanced in an average of 9.6-fold that of curcuminoid suspension. It was concluded that the bioavailbility of curcuminoid was enhanced greatly by the microemulsion.

KEYWORDS: *curcuminoid, bisdemethoxycurcumin, microemulsion, physicochemical properties in vitro, peroral bioavailability*

INTRODUCTION

Curcuminoid, a dietary polyphenolic compound isolated from turmeric, the rhizomes of *Curcuma longa* Linn, is a mixture of three bis-α,β-unsaturated-γ-diketone hydrophobic polyphenols named curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC). Their chemical structures are illustrated in Figure 1. For a long history, curcuminoid has been used as a coloring and flavoring agent worldwide. Interest in this dietary polyphenol has grown in recent years due to its vast array of beneficial pharmacological effects including antivirus, antioxidation, anti-inflammatory, anti-Alzheimer's disease, and anti-HIV activities.[1−3] Curcuminoid is considered as a novel, safe, and promising anticancer agent for both prevention and treatment of cancer. It can inhibit cancer cell invasion *in vitro* and *in vivo,* suggesting mechanism is by regulation of invasive gene such as ECM degradation enzymes (MMP-9, MT1-MMP, MMP-2). The potency order of the three curcuminoids for inhibition of cancer cell invasion is BDMC > DMC > curcumin.[4−6] Phase I clinical trials indicated that curcuminoid is safe for human even at an oral dosage of 12 g per day, and the U.S. National Cancer Institute has chosen it as a third generation cancer chemopreventive agent.[7]



curcumin	R_1=OCH_3	R_2=OCH_3
DMC	R_1=OCH_3	R_2=H
BDMC	R_1=H	R_2=H

Figure 1. Chemical structures of curcumin, DMC, and BDMC.

Clinical advancement of this promising molecule has been hindered by its poor water solubility, short biological half-life, and low bioavailability following oral administration.[8,9] Recently, several methods have been developed to improve the solubility and oral bioavailability of curcumin including adjuvant with piperine,[10] phospholipid complexation,[11] the complexation with soy protein isolate,[12] solid dispersions with polyvinylpyrrolidone (PVP),[13] nanoparticle,[14,15] nanoemulsion,[16] and liposome encapsulation.[17] Whereas these techniques increase the oral bioavailability of curcumin, piperine's effect on the metabolism of other drugs, the hygroscopic nature of PVP, and the complicated process of complexation and encapsulation are most likely to limit their practical utilization.

Microemulsion, i.e., nanoemulsion, consisting of surfactant, cosurfactant, oil, and water is defined as a colloidal, optically isotropic, transparent or slightly opalescent formulation,[18,19] and the mean droplet radius of that is between 10 and 100 nm, which has several advantages for pharmaceutical use, such as ease of preparation, long-term stability, and high drug solubilization capacity. Microemulsion is suitable for the incorporation of poorly water-soluble drugs to improve oral absorption,[20,21] and in the field of functional food, microemulsion has many applications in solving the problems of solubility as well as stability of nutraceuticals and food additives.

The main purpose of this study is to design, prepare, and characterize a curcuminoid-loaded microemulsion (Cur-ME) formulation to improve its oral bioavailability. On the basis of a

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solubility study and pseudoternary phase diagrams, Cur-ME was developed after screening oils, surfactants, and cosurfactants, and *in vitro* characterized by studying the morphology, particle size, zeta potential, pH, viscosity, and surface tension. Oral absorption of Cur-ME in rats was assessed. In previous studies, curcumin was usually chosen as a representative composition to study the enhanced bioavailability of curcuminoid.[11,22,23] However, no pharmacokinetcis of BDMC has been reported yet for the microemulsion formulation, which may be due to its low loading capacity of BDMC or the lack of a sensitive analytic method for BMDC. Due to the potency of BDMC for inhibition of cancer cell invasion is the best among of curcuminoid, and BDMC has the highest bioavailability among of curcuminoid; therefore, it is necessary that BDMC is treated as the representing component to evaluate the oral bioavailability of Cur-ME in rats. In this study, we evaluate the pharmacokinetics of BMDC in rats after oral administration of Cur-ME by HPLC with fluorescence detector.

MATERIALS AND METHODS

Materials. Curcuminoid, as a mixture of curcumin, BDMC, and DMC (99%), was purchased from Aladdin Reagent Co., Ltd. (Shanghai). Medium chain triglycerides (Labrafac Lipophile WL 1349, WL1349), medium chain triglycerides EP (Labrafac CC), and purified diethylene glycol monoethyl ether (Transcutol HP) were donated from Gattefosse (France). Caprylic/capric triglycerides (ODO) were purchased from Zhejiang Qiandao Final Chemical Co. Ltd. (Zhejiang, China). Propylene glycol dicaprylate/dicaprate (Miglyol 840), polysorbate 80 (Tween 80), and polyethyleneglycol 400 (PEG 400) were obtained from Sasol (Germany). Polyoxyethylene castor oil (Cremorphor EL) and polyoxyethylene hydrogenated castor oil (Cremorphor RH40) were obtained from BASF (Germany). Glycerine was purchased from Beijing J&K Chemical Technology Ltd. (Beijing, China). Methanol and acetonitrile were of HPLC grade. Double-distilled water was used throughout the study. All other chemicals and solvents were used without further purification.

Preparation of Curcuminoid-Loaded Microemulsion. *Solubility Study.* The solubility of curcuminoid, BDMC, in the vehicles was determined by the shake flask method. Briefly, an excess of curcuminoid was added individually to the oils, surfactants, and cosurfactants (1 g each) in screw capped tubes. Mixtures were then vortexed at high speed for 5 min and shaken for 72 h in water bath shaker maintained at 25 °C. After 72 h, each sample was centrifuged at 12 000 rpm for 15 min; aliquot of supernatant was diluted with methanol. The amount of curcuminoid dissolved in the vehicles was determined by HPLC (Shimadzu LC-10AT, Japan, μBondapak C18 column (150 mm × 4.6 mm, 5 μm); the mobile phase (acetonitrile/5% acetic acid in water in the ratio of 50/50) was run at a flow rate of 1 mL/min, and curcuminoid was detected at 420 nm. Solubility studies were carried out in triplicate.

Construction of Pseudoternary Phase Diagrams. Pseudoternary phase diagrams containing oil−surfactant/cosurfactant−water were constructed using the water titration method. Mixtures (systems A−E) of the oil phase containing WL1349 with the surfactant phase including a combination of surfactant and cosurfactant, cremophor RH40/glycerine, 1:1 (system A); cremophor EL:PEG 400, 1:1 (system B); cremophor RH40:glycerine, 3:1 (system C); cremophor RH40:glycerine, 2:1 (system D); cremophor RH40:glycerine, 1:2 (system E), were prepared at certain weight ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10. The mixtures of the oil phase and surfactant phase of 11 different weight ratios were accurately weighed into 11 glass tubes. The mixtures in each tube were mixed homogeneously using a vortex mixer until the oily liquid mixtures were obtained at room temperature. Water was then added drop-by-drop using a dropper into each oily mixture. During the titration, samples were stirred vigorously for a sufficient length of time for homogenization and visually monitored against a dark background by illuminating the samples with white light. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transition occurred was derived from the weight measurements. These values were then used to determine the boundaries of the microemulsion regions corresponding to the selected optimum ratios of combination vehicles for developing Cur-ME.

Formulation and Preparation of Cur-ME. On the basis of the pilot studies (equilibrium solubility, pseudoternary phase diagram, and supersaturation studies), WL 1349, cremorphor RH40, and glycerine were used as the oil, surfactant, and cosurfactant, respectively. Five formulations of Cur-ME (ME1-ME5, Table 1) were prepared containing a fixed proportion of curcuminoid (based on BMDC, 0.5% w/w) dissolved in a mixture (33%, w/w) of WL 1349, cremorphor RH40, glycerine, and water (67%, w/w) according to the results of pseudoternary phase diagrams. A typical formulation (e.g., ME2) was prepared following the previously reported methods[22] with slight modifications. Briefly, 400 mg of WL 1349, 450 mg of cremorphor RH40, 150 mg of glycerine, and 50 mg of curcuminoids powder were placed in a vial, and mixed at 25 °C with a magnetic stirrer until an isotropic mixture formed under light shielding. Then, 2 mL of double-distilled water was added to the mixture drop by drop, and undissolved drug was filtered through 0.45 μm membrane. After appropriate dilution with methanol, the concentration in the filtrate was measured by HPLC.

Table 1. Solubility of Curcuminoid in Various Vehicles at 25 °C (n = 3)[a]

vehicle	solubility (mg/g) or mL
Oils	
ODO	9.39 ± 0.24
labrafac CC	8.21 ± 0.64
IPP	9.18 ± 0.32
oleic acid	1.39 ± 0.030
WL 1349	12.60 ± 0.20
miglycol 840	11.12 ± 0.82
Surfactants	
cremphor EL	54.76 ± 2.89
tween 80	67.25 ± 4.25
cremphor RH40	86.27 ± 3.01
Cosurfactants	
transcutol P	66.66 ± 1.20
PEG 400	95.07 ± 4.50
propylene glycol	11.89 ± 2.03
glycerine	3.95 ± 0.12

[a]All values reported are means ± SD (n = 3).

Determination of the Curcuminoid Content in the Microemulsion. The content of curcuminoid (based on BDMC) in the microemulsion was determined as follows. Briefly, 1 mL of the microemulsion was accurately added to a 10 mL volumetric flask containing 7 mL of methanol. After being ultrasonic extracted for 15 min, the solution was diluted with methanol to 10 mL and then filtered using 0.45 μm cellulose nitrate membrane. The filtrate was diluted 50-fold with mobile phase, and a 20 μL aliquot was injected into above HPLC system (Shimadzu LC-10AT, Japan). Results showed that BDMC could be completely separated from curcumin and DMC under analytical conditions (Figure 2).

Characterization of Cur-ME. *Morphological Evaluation.* The morphology of Cur-ME was investigated by transmission electron microscope (TEM, Hitach H7650, Japan). The sample was prepared by depositing a drop of diluted microemulsion samples onto a film-coated copper grid, later staining it with a drop of 2% aqueous solution of phosphotungstic acid, and allowing it to dry at room temperature before the examination.

Droplet Size, Zeta Potential, and pH Value Measurement. The average droplet size, polydispersity index, and zeta potential of Cur-

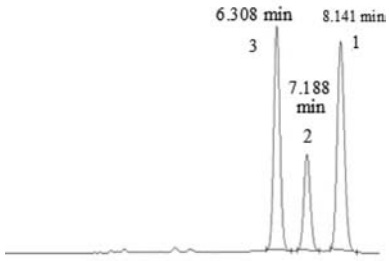

Figure 2. HPLC-UV profile of curcuminoids: (1) curcumin, (2) demethoxycurcumin (DMC), and (3) bisdemethoxycurcumin (BDMC).

ME were determined by the Zatasizer 3000HSA Measurement (Malvern Instruments Ltd. U.K.). The pH value of the sample was measured by a pH meter (model PHS-2C, Lida equipment mill, Shanghai, China) at 20 °C. All measurements were carried out in triplicate.

Surface Tension Measurement. Surface tension of Cur-ME was measured by DCAT 2.1 tensiometer (Dataphysics, Germany) using Wilhemy's plate method. A square platinum plate was washed twice with distilled water, and heated in a reductive flame to purge all impurities. This cleaning procedure was repeated before every measurement. During the measurement the plate is dipped into the liquid. The tensiometer measures the pulling force of the liquid on the plate and calculates the surface tension with the given plate size.

Viscosity. The viscosity of Cur-ME was analyzed by a Rheometer (DV-III, Brookfield). The microemulsion was placed in a cone-and-plate viscometer and maintained at 25 °C. Viscosities were detected at 50/s shear rate with a No. 1 rotor. After the level stabilized for 30 s, the data were recorded. Reproducibility (triplicate) was checked for the samples, and no significant differences (±SD) were observed.

In Vivo Absorption of Cur-ME in Rats. The study was approved by the Ethical Committee of China Pharmaceutical University. There were 18 male Sprague−Dawley rats (body weight 200−250 g) divided randomly into three groups. They fasted for 12 h but were allowed to take water freely. Curcuminoid suspension (curcuminoid powder dispersed in 0.5% sodium carboxymethycellulose, CMC-Na solution)

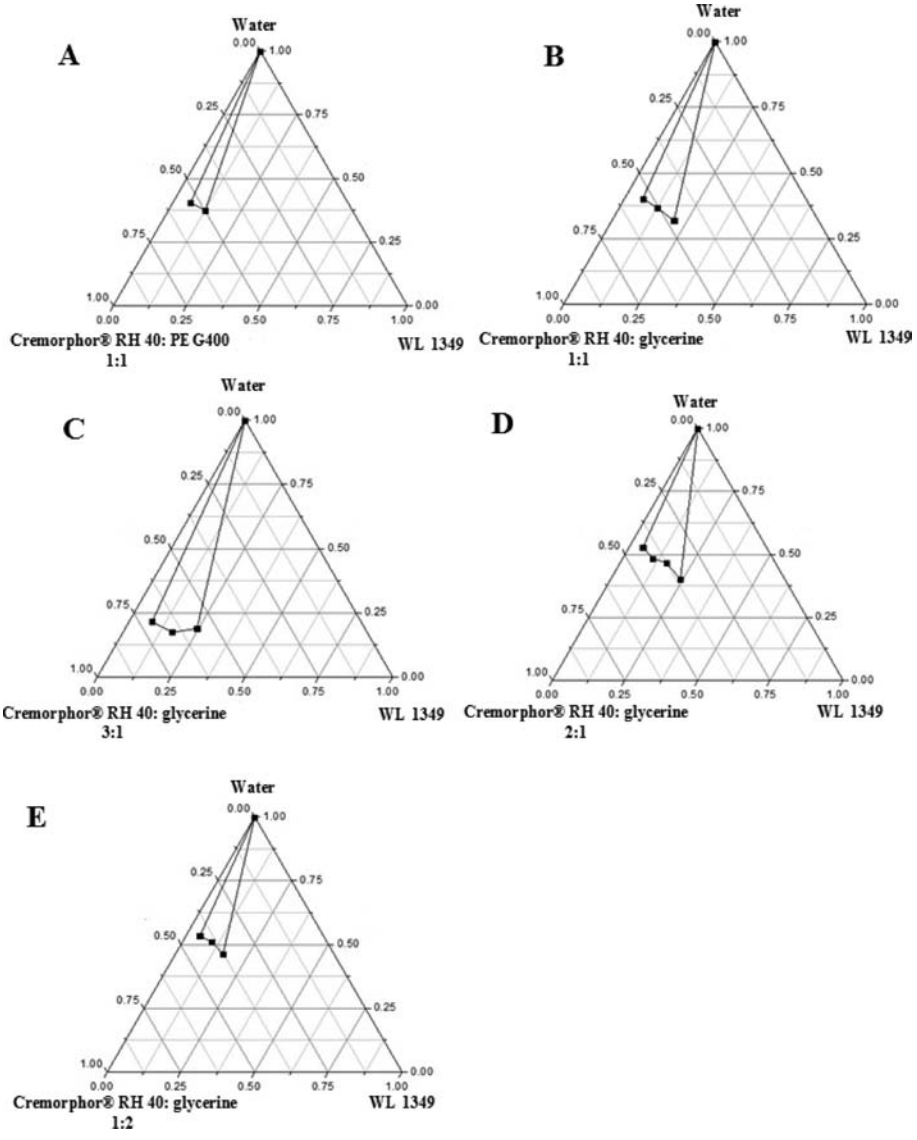

Figure 3. Pseudoternary phase diagrams composed of the oil phase (i.e., WL 1349) and various surfactants. The surfactant phase was as follows: (A) cremorphor RH 40:PEG400 (w/w) = 1:1; (B) cremorphor RH 40:glycerine (w/w) = 1:1; (C) cremorphor RH 40:glycerine (w/w) = 3:1; (D) cremorphor RH 40:glycerine (w/w) = 2:1; (E) cremorphor RH 40:glycerine (w/w) = 1:2.

Table 2. Composition of Cur-ME Formulations ME1-ME5 Containing 50 mg of Curcuminoid in 3000 mg of a Mixture of an Oil Phase and Surfactant Phase (33%, w/w) and Water (67%, w/w)

composition	formulation (mg)				
	ME1	ME2	ME3	ME4	ME5
curcuminoid	50	50	50	50	50
Oil Phase					
WL 1349	500	400	300	200	100
Surfactant Phase					
cremorphor RH40	375	450	525	600	675
glycerine	125	150	175	200	225
water	2000	2000	2000	2000	2000
droplet size[a](nm)	112.31 ± 8.62	51.24 ± 3.83	45.72 ± 1.43	36.24 ± 0.82	18.63 ± 0.43
BDMC content[a](mg/mL)	11.33 ± 1.56	12.12 ± 2.87	12.66 ± 3.42	13.03 ± 1.87	13.09 ± 2.64

[a]All values reported are means ± SD (n = 3).

and Cur-ME (ME2 and ME5) which were equivalent to 24 mg/kg of BDMC were given to rats by intragastric administration, respectively.

About 500 μL of blood samples were collected from eyeground veins at 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, and 6 h. The plasma obtained after centrifugation (15 min, 4000 rpm) was immediately stored at −20 °C until it was analyzed. A 100 μL sample of plasma was transferred to a 1.5 mL polyethylene centrifuge tube, and then mixed with cold acetonitrile (100 μL) for 3 min. The precipitate of denatured proteins was separated by centrifugation at 12 000 rpm for 10 min. An aliquot (20 μL) of supernatant was directly injected into a HPLC system as above, and determined at ex = 436 nm, em = 518 nm by fluorescence detector.

The method was validated by adding various quantities of curcuminoid (based on BDMC) to blank rat plasma. Resulting concentrations of BDMC were 2.04, 4.08, 8.16, 16.32, 32.64, 65.28, and 102 ng/mL. These calibrations were subjected to the entire analytical procedure, as well as to validate the linearity, precision, and accuracy of the method.

Pharmacokinetic Data Analysis. The peak plasma concentration (C_{max}) and the time of peak plasma concentration (T_{max}) were directly obtained from the experimental points. All the other pharmacokinetic parameters were computed by software program DAS2.0. The relative bioavailability (F) was calculated using the following equation:

$$F(\%) = \frac{\mathrm{AUC}_{test}}{\mathrm{AUC}_{reference}} \times 100$$

Here, AUC_{test} is the area under the curve after oral administration of the Cur-ME, and $\mathrm{AUC}_{reference}$ is the area under the curve after oral administration of curcuminoids suspension.

One-way analysis of variance (ANOVA) was applied to compare data from different formulations. All data were expressed as the mean ± standard deviation (SD), and p-value <0.05 was considered significant.

RESULTS AND DISCUSSION

Preparations of Cur-ME. Appropriate vehicles should have good solubilizing capacity of the drug, which is essential for allowing the presentation of the drug in solution. The solubility of curcuminoid in various oils, surfactants, and cosurfactants is shown in Table 1. The results showed that the solubility of curcuminoid in all vehicles was more compared to that in water. For all tested oils, WL 1349 gave the highest solubility of curcuminoid with 12.60 ± 0.20 mg/g, followed by miglycol 840 and ODO with 11.12 ± 0.82 and 9.39 ± 0.24 mg/g, respectively. Hence, WL1349 was selected as the oil phase for further investigations. Among various surfactants screened, cremophor RH40, a nonionic hydrophilic surfactant with an HLB of 15, exhibited the highest solubilizing potential for curcuminoid with 86.27 ± 3.01 mg/g, followed by tween 80 and cremophor EL with 67.25 ± 4.2 and 54.76 ± 2.89 mg/g, respectively. Therefore, cremorphor RH40 was fully considered as the surfactant for further investigations.

Pseudoternary phase diagrams containing oil−surfactant/cosurfactant−water were constructed in the absence of curcuminoid to identify the microemulsion regions and to optimize the concentration of the selected vehicles. In this experiment, transcutol P, glycerine, polyethylene glycol 400 (PEG 400), and propylene glycol as cosurfactant, respectively, were investigated with WL 1349 as the oil phase and cremorphor RH40 as the surfactant. Only PEG 400 and glycerine as cosurfactant, respectively, could form microemulsion, and yet, the area of the microemulsion region in the pseudoternary phase diagrams when PEG 400 was used as cosurfactant was smaller than that when glycerine was used as cosurfactant (Figure 3A,B). Therefore, glycerine was used as the desirable cosurfactant.

K_m (the weight ratio of the surfactant to the cosurfactant) was considered as one of the key factors on affecting the area of the microemulsion regions. In this study, the pseudoternary phase diagrams, containing WL 1349−cremorphor RH40/glycerine−water with K_m fixed at 3:1, 2:1, 1:1, 1:2, respectively, in the absence of curcuminoid were described in Figure 3 (Figure 3C,D,B,E). The area of the microemulsion region increased as K_m increased, and when K_m was set at 3:1, the maximum microemulsion region was attained. Thus, K_m was set at 3:1 for further studies.

Some basic guidelines need to follow to optimize the microemulsion formulation such as safety, compatibility, drug solubility, droplet size, and the stability of the formed microemulsion, etc. Therefore, we prepared many kinds of Cur-ME according to the concentration of components for the existing region of the microemulsions in the pseudoternary phase diagrams. The solubility of curcuminoid in each microemulsion and droplet size were determined (Table 2). Results showed that the drug-loading capacity of the formulation ME1 was smaller in comparison with others. Among the microemulsion formulations ME2, ME3, ME4, and ME5, the solubility of curcuminoid was increased with the ratio of surfactant to oil. When the ratio of surfactant to oil ranged from 6:4 to 9:1, the solubility of curcuminoid in the microemulsion ranged from 12.12 ± 2.87 to 13.09 ± 2.64 mg/mL, and yet, no distinct differences on the drug-loading capacity among the four kinds of microemulsion were observed and droplet sizes were under 100 nm. As is well-known, the surfactant and the cosurfactant can enhance the intestinal absorption of drugs, and the greater the amounts of the

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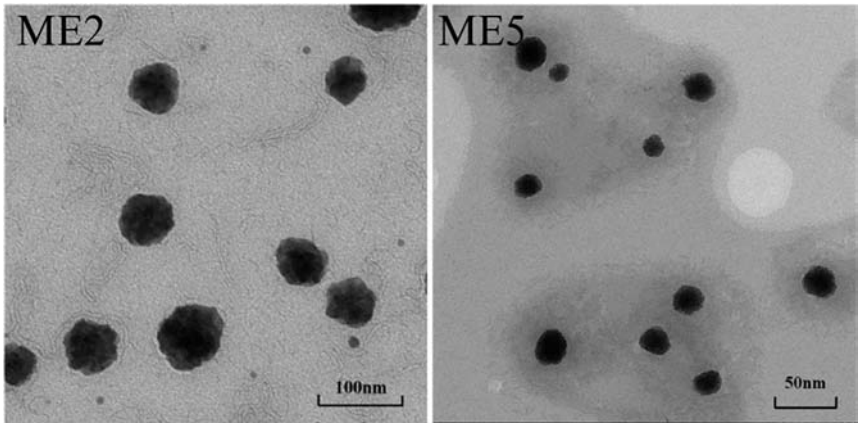

Figure 4. Transmission electron micrographys of the Cur-ME (ME2 and ME5) (×20 000).

Table 3. Parameters of Physicochemical Characteristics of the Selected Cur-ME (n = 3)

formulation	mean droplet size (nm)	polydispersity index	zeta potential (mV)	pH	surface tension ($\times 10^{-3}$N/m)	viscosity value (mPa s)	solubility of drug (mg/mL)
ME2	51.24 ± 1.45	0.14 ± 0.030	−4.17 ± 0.53	6.43 ± 0.040	30.75 ± 0.029	30.59 ± 5.31	10.59 ± 0.19
ME5	18.62 ± 0.51	0.11 ± 0.030	−4.23 ± 0.63	6.21 ± 0.020	40.21 ± 0.030	23.40 ± 3.91	12.01 ± 0.23

surfactant and the cosurfactant, the smaller droplet size of the microemulsion, which is beneficial to the absorption of the drug. However, few studies on the effect of the oil amount in the microemulsion on the physicochemical properties and *in vitro* and *in vivo* pharmacokinetics of the microemulsion were investigated. Therefore, we selected two kinds of Cur-ME formulations, ME2 and ME5, with the same amount of surfactant and different amount of the oil for further studies. ME2 (40:45:15, w/w/w) and ME5 (10:67.5:22.5, w/w/w) were formed by WL 1349 (oil), cremorphor RH40 (surfactant), and glycerine (cosurfactant).

Characterization of Cur-ME. The appearance of ME2 and ME5 were both uniform and translucent with a clear orange-yellow opalescence. The TEM picture suggested that the droplet of ME2 and ME5 appeared in perfect round shape without aggregation (Figure 4). The other physicochemical characteristics of ME2 and ME5 appear in Table 3. The results showed that the amount of oil in the microemulsion had great effects on the droplet size, viscosity, and surface tension of the microemulsion. When the amount of oil in the microemulsion ranged from 10% to 40% (w/w), the droplet size and viscosity of the microemulsion increased from 18.6 to 51.2 nm, and 23.4 mPa s to 30.59 mPa s, respectively, and yet, the surface tension of the microemulsion decreased from 40.21 × 10^{-3} N/m to 30.75 × 10^{-3} N/m. Many studies showed that curcuminoid decomposed in a pH-dependent manner, with faster reactions at neutral to basic conditions, and they are known to be stable at a pH below 6.5. The pH values of ME2 and ME5 were 6.43 and 6.21, respectively, which was beneficial to the stability of the drug, and the pH of the microemulsion had no effect with the amount of oil. We cannot optimize Cur-ME formulation by the results of the physicochenmical properties, and thus, ME2 and ME5 were chosen for oral bioavailability test in rats for further investigations.

In Vivo Studies in Rats. It has been reported that natural curcuminoid is a mixture of curcumin, DMC, and BDMC in a ratio of 77:17:3.[24] However, our previous studies have shown that commercially available curcuminoid obtained from Aladdin Reagent Co., Ltd., contains ∼59% pure curcumin, 12% DMC, and 30% BDMC (w/w). Despite the pharmacokinetics of curcumin having been extensively studied, the pharmacokinetics of BDMC remains largely unexplored. This could be due to its relatively low abundance in the commercial curcuminoid or lack of a sensitive analytic method for BMDC. However, the potency of BDMC for inhibition of cancer cell invasion is the best among of curcuminoid, and BDMC had the highest oral bioavailability among curcuminoid;[4−6] thus, BDMC was chosen, in this study, as a representative composition to evaluate oral bioavailability of Cur-ME in rats.

Previously, a liquid chromatography coupled with tandem mass spectrometric (LC-MS/MS) method has been developed for the determination of BDMC in biological fluids.[25,26] Because BDMC had the characteristics of fluorescence absorption, a HPLC technique with the fluorescence detector was used to detect the concentration of BDMC in the plasma in this study. Under the chromatographic conditions described above, optimized separation and detection conditions were achieved in plasma. The retention time of BDMC is shown in Figure 5 at about 8.1 min. The detection limit for BDMC at a signal-to-noise ration of 3:1 was 0.5 ng/mL in plasma.

The calibration curve of BDMC in rat plasma was linear in the range 2.04−102 ng/mL plasma. Using the linear least-squares regression, the calibration line of BDMC was $y = 1 \times 10^3 x + 25\,492$ with $r = 0.9991$. The mean relative recoveries of BDMC at high, middle, and low concentrations ranged from 86.29% to 97.32% in plasma. Both intra- and interday precision

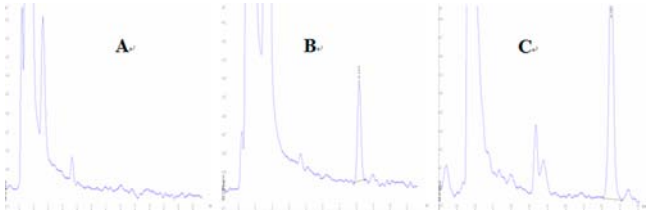

Figure 5. Representive HPLC−fluorescence profiles of blank plasma (A), blank plasma containing BDMC (B), and plasma after oral adminstration of the Cur-ME (C).

(expressed as percent relative standard deviation, RSD%) of BDMC were within 10.0% in plasma. The intra-and interday accuracy (expressed as a percent of the nominal value) ranged from 89.34% to 98.56% in plasma. Therefore, it was found that recoveries and intra- and interday RSD of BDMC in rat plasma were satisfying.

An *in vivo* absorption study was undertaken to determine whether or not the enhanced solubility of curcuminoid could increase the GI absorption of the drug and screen the optimized Cur-ME formulation. Figure 6 shows mean plasma concentration−time curves of BDMC in rats after oral adminstration of Cur-ME (ME2 and ME5) equivalent to 24 mg/kg of BDMC, respectively (mean ± SD, n = 6).

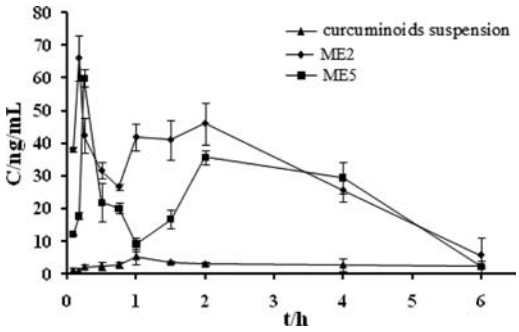

Figure 6. Mean plasma concentration−time curves of BDMC in rats after oral adminstration of curcuminoids suspension and the Cur-ME (ME2 and ME5) equivalent to 24 mg/kg of BDMC, respectively (mean ± SD, n = 6).

concentration−time curves of BDMC in rats after oral administration of Cur-ME (ME2 and ME5) and curcuminoid suspension equivalent to 24 mg/kg of BDMC (n = 5), respectively. From the profile, we could know that the concentration−time curves of BDMC after oral administration of Cur-ME and curcuminoid suspension were much different. The average value of C_{max} was 5.39 ng/mL after oral administration of curcuminoid suspension with a T_{max} of about 1 h; however, there were obviously double absorption peaks on the mean plasma concentration−time profile of Cur-ME. The average values of C_{max} after oral administration of ME2 were 66.19 and 46.72 ng/mL with a T_{max} of about 0.167 and 2 h, respectively. However, the average values of C_{max} after oral administration of ME5 were 59.92 and 35.68 ng/mL with a T_{max} of about 0.25 and 2 h, respectively. The speculated reason may be that, after oral administration of Cur-ME, BDMC was metabolized in the hepatic microsomes to be BDMC-glucuronic acid conjugate with improved water-solubility, and the metabolites were discharged into the intestine with the bile secretion. Under the role of intestinal bacterial enzyme, the metabolites were hydrolyzed into protorype drugs, and then were again absorbed into the liver, showing the phenomenon of enterohepatic circulation and the concentration−time curve bimodal. All the other parameters were obtained by the software program DAS2.0 (Table 4). As shown in Table 4, the relative bioavailabilities of BDMC in rats after oral administration of ME2 and ME5 were enhanced by about 9.6-fold and 7.53-fold, respectively, compared with that of curcuminoids suspension, indicating significant improvement of the drug absorption by microemulsion formulations. Generally, droplet size of the microemulsion is a crucial factor because it determines the rate and extent of drug release as well as absorption. The results obtained from the *in vitro* characteristics confirmed that the droplet size of ME2 was larger than that of ME5; however, the oral bioavailability of BDMC in rats after oral administration of ME2 was higher than that after oral administration of ME5. It showed that the amount of the oil in the microemulsion could affect the oral absorption of the drug, and the greater the amount of oil in the microemulsion, the higher the oral bioavailability of the drug, when it existed within the microemulsion region. Wang et al. reported that WL 1349 could be emulsified by deoxycholate, and then was hydrolyzed to fatty acids and monoglycerides by pancreatic lipase rapidly, enhancing oral absorption of hydroxysafflor yellow A.[27] Therefore, we speculated the greater the amount of WL1349 was, the more the absorbed drug was. Finally, ME2 was chosen as the optimized Cur-ME formulation.

Table 4. Pharmacokinetic Parameters of BDMC in Rats after Oral Adminstration of Curcuminoid Suspension and the Cur-ME (ME2 and ME5) Equivalent to 24 mg/kg of BDMC, Respectively (Mean ± SD, n = 6)

param	curcuminoid suspension	ME2	ME5
MRT/h	2.92 ± 0.12	2.19 ± 0.075	2.58 ± 0.13
AUC_{0-t}/ (ng h /mL)	18.66 ± 1.54	180.97 ± 2.71[a]	140.58 ± 8.31[a]
C_{max1}/(ng/mL)	5.39 ± 0.13	66.19 ± 4.43[a]	59.92 ± 2.73[a]
C_{max2}/(ng/mL)		46.72 ± 3.64	35.68 ± 2.98
F_{0-12h} (%)		969 ± 124	753 ± 193

[a] $p < 0.01$ vs curcuminoids suspension.

The reasons for poor bioavailability of this agent within the body include the following:[28,29] (1) There is poor absorption which is due to the low solubility of curcumin in water or in the gastrointestinal tract. 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) There is a 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. (3) Systemic elimination or clearance of curcumin from the body is also an important factor, which determines its relative biological activity.

The oral bioavailabilities of many poorly water-soluble drugs have been improved by the microemulsion employing a single or combined mechanism. One of the main reasons for the enhanced drug oral bioavailability by the microemulsion is the excellent efficiency of the microemulsion in improving the drug solubility and increasing the dissolution rate. After oral administration, the microemulsion provides ultralow interfacial tensions and large o/w interfacial areas, resulting in the incorporation of poorly water-soluble pharmaceuticals inside the fine oil droplets. The large specific surface area of the fine oil droplets also enables a more efficient drug transport through the intestinal aqueous boundary layer, leading to an improvement in oral bioavailability. The microemulsion might be absorbed by lymph circulation, which avoided the hepatic first pass effect, and was thought to be another factor of increasing the oral bioavailability of curcuminoids. The superior performance of Cur-ME in oral absorption might also be attributed to increasing membrane fluidity and permeability in intestinal epithelial cells. These mechanisms need further investigation and confirmation.

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Notes

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ABBREVIATIONS USED

DMC, demethoxycurcumin; BDMC, bisdemethoxycurcumin; Cur-ME, curcuminoid-loaded microemulsion; K_m, the weight ratio of surfactant and cosurfactant; HPLC, high performance liquid chromatography; TEM, transmission electron microscope; C_{max}, peak plasma concentration; T_{max}, time of peak plasma concentration; AUC, area under the curve; MRT, mean retention time; *F*, relative bioavailability

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