Silica-Lipid Hybrid Microparticles as Efficient Vehicles for Enhanced Stability and Bioaccessibility of Curcumin

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SUMMARY

Curcumin is an active ingredient with multiple functions, but its application is often restricted due to its poor water-solubility, weak stability, and consequently low bioaccessibility. Based on this, the aim of this work was to develop a new vehicle to overcome the restrictions above. In the current research, the curcumin-loaded nanoemulsion and subsequently curcumin-loaded silica-lipid hybrid microparticles were developed through the emulsification process and vacuum drying method, respectively. The loading content of curcumin in nanoemulsion and microparticles were measured to be (0.30±0.016) and (0.67±0.019) %. FTIR and XRD analysis of microparticles revealed that curcumin was encapsulated in porous silica with a XRD-amorphous form. In vitro antioxidant activities showed that the encapsulation would not affect the antioxidant activities of curcumin. In vitro simulated digestion indicated that nanoemulsion and microparticles had higher bioaccessibility compared to the control group. The storage stability results showed that the nanoemulsion and microparticles could maintain

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the stable states at 4, 25 and 40 °C for 6 weeks in dark. Moreover, the microparticles had a better chemical stability than nanoemulsion under the light. The cell viability was over 80 % when the concentration of nanocarriers was less than 45 μg/mL. Hence, the microparticles could be a promising strategy to load curcumin and improve its solubility, light stability as well as the bioaccessibility.

**Key words:** curcumin, silica-lipid hybrid microparticles, antioxidant activity, bioaccessibility, stability

**INTRODUCTION**

Curcumin is extracted from the rhizome of Curcuma longa, which has extensive pharmacological and biological activities. The medical value of curcumin is reflected on the treatment of various inflammation and some other diseases for centuries in indigenous medicine (1). In recent years, a number of researchers have focused on curcumin and it has been well-documented that curcumin possesses antioxidant (2), antimicrobial, anti-inflammatory and anticancer activities (2-4). Meanwhile, a large amount of reports has confirmed the potential of curcumin in the treatment of diabetes, Rheumatoid arthritis (RA), neurodegenerative diseases and cardiovascular diseases (5-7). Furthermore, curcumin is approved as a “Generally Regarded as Safe” compound by the U.S. Food and Drug Administration (8), implying the potential value in food and health care products because of the high-security and biological activities.

Despite a variety of advantages, curcumin has difficulty in practical applications on account of its low water solubility, chemical instability and rapid metabolism in the gastrointestinal tract (8,9). These shortcomings would ultimately lead to inadequate absorption and low bioavailability of curcumin. Aiming at these problems, many strategies have been developed which include chemical/physicochemical and physical-mechanical methods (10). Among these methods, the lipid-based nanotechnology delivery systems are widely recognized due to their advantages and application prospect. The most common types of
nanoparticles include nanoemulsion (NE), solid-lipid nanoparticles (SLN), nanostructure lipid carriers (NLC), liposomes, self-emulsifying drug delivery systems (SEDDS) and so on. These nanoparticles with small particle size can improve the water-solubility and bioaccessibility of curcumin (11). Besides, the nanocarriers can encapsulate the curcumin molecules inside the cavities so as to protect curcumin from degradation and improve the stability in varying degree (12). Enormous studies have displayed that the nanocurcumin has higher antioxidant and antimicrobial activities than curcumin raw material (13,14). Furthermore, other activities of curcumin such as anticancer activity should not be affected. Researchers have found that liposomal curcumin could suppress the growth of pancreatic carcinoma and demonstrated antiangiogenic effects in vivo (15). And another research reveals that curcumin-SLN have xenografts-targeting effect and enhance the inhibition efficiency to tumor significantly from 19.5 to 69.3 % (16). These results indicate that the activity of nano-curcumin is tantamount to or better than curcumin raw material.

Although these lipid-based nanoparticles have many advantages, they still face the challenges of physical stability and long-term storage stability. Solidification of liquid formulation, such as “dry emulsion”, is considered a promising approach to solve these problems, which can improve the storage convenience and physicochemical stability during long-term storage. Spray drying (17), lyophilisation (18), vacuum drying (19) and physical adsorption (20) are the common solidification methods to eliminate the water phase in the liquid emulsion. Silica-lipid-hybrid (SLH) microparticles is a kind of solid system based on silica particles and oil-in-water emulsion, which were reported for the first time by Simovic et al. (21) and Tan et al. (22). The researches show that the SLH microparticles significantly improve the solubility and stability of the model water-insoluble drugs, and also enhance the oral absorption of drugs in experimental animals by simulating food effects (21-23). In another study, researchers find that the ibuprofen-SLH microparticles prepared by spray drying can produce higher drug solubilisation compared to the control group during a two-step dissolution and the bioavailability of microparticles show a nearly 1.95-fold increased for ibuprofen-SLH microparticles with reference to the commercial tablets in clinical trials. Furthermore, the safety
assessments reveal negligible acute side effects in connection with the blank and ibuprofen-SLH formulation (24). These results indicate that microparticles possess oral safety and effectiveness, which is a promising method to improve the oral absorption of water-insoluble substance.

Various methods have been advanced to improve the water-solubility and bioavailability of curcumin in recent years, but few research has investigated the effect of curcumin-loaded silica-lipid hybrid microparticles. In the current study, curcumin nanoemulsion was developed and the optimum formulation was selected through orthogonal experiment. Then the microparticles was prepared on basis of nanoemulsion through a two-step process by vacuum drying. The physicochemical properties, antioxidant abilities, bioaccessibility, lipid digestion properties, and storage stabilities of the two samples were examined to investigate the similarities and differences between microparticles and nanoemulsion. These studies will provide a new perspective to solve the problems of curcumin and promote its application in more fields.

MATERIALS AND METHODS

Materials

Curcumin (95 %) was purchased from Sciphar Natural Products Co. Ltd. (Shanxi, China). Octyl and decyl glycerate (ODO) was obtained from Henan Zhengtong Food Technology Co. Ltd. (Henan, China). Tween 80 and Tween 60 were provided by Guangzhou Runhua Chemical Co. Ltd. (Guangzhou, China). Phosphatidylcholine 60 (PC 60) was produced by Aikang Fine Chemical Co. Ltd. (Shanghai, China). Hydrophilic fumed silica (Aerosil 380) was provided by Evonik Degussa (Germany). Povidone K30 was purchased from Chongqing Star-Tech & JRS Specialty Products Co. Ltd. (Chongqing, China). Carboxymethylcellulose sodium (CMC-Na) was obtained from Yuhe Food Additive Co. Ltd. (Zhengzhou, China). All the ingredients were of food grade.

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was provided by Tokyo Chemical Industry (Japan). 1,1,3,3-Tetraethoxypropane (97 %) was purchased from Shanghai Macklin
Biochemical Co. Ltd. (Shanghai, China). Phosphotungstic acid, potassium bromide (KBr), trichloroacetic acid (TCA) and 2-thiobarbituric acid (≥98.5 %) were from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Pepsin, pancreatin and bile extract were from Sigma Chemical Co. Ltd. (USA). DMEM, Fetal Bovine Serum (FBS) and penicillin-streptomycin was purchased from Cellmax cell technology Co. Ltd. (Beijing, China).

All other chemicals used were of analytical grade with no further purification.

Preparation of curcumin nanoemulsion

Certain mass fractions of Tween 80 (2.0-5.0 %), Tween 60 (2.0-5.0 %) and phosphatidylcholine 60 (PC 60) (2.0-5.0 %) were dissolved in 10 % ODO at 70 °C, followed by the addition of 0.3 % curcumin raw material. 0.6-1.2 % Povidone K30 (PVP K30) was added to the oil phase after curcumin was completely dissolved. Then, Milli-Q water (73.5-83.1 %) was added to the oil mixture as the continuous phase. The mixture was stirred for 10 min to form o/w emulsion.

Determination of formula

The formula of curcumin nanoemulsion was optimized by orthogonal experiment designed in Table 1 with 5 factors: the temperature (°C) of preparation (A) and the mass fraction in the formula of Tween 80 (B), Tween 60 (C), PC 60 (D) and PVP K30 (E), respectively. Emulsion stability index (ESI) was chosen as evaluation index (25). The emulsion was stored at room temperature for 48 hours after preparation, and then the data was measured to calculate ESI by the formula as follows:

$$ESI = \left(1 - \frac{h_C + h_S}{h_E}\right) \cdot 100$$

where $h_E$ is the total height of emulsion, $h_C$ is the height of creamed phase and $h_S$ is the height of sedimentation phase.
Preparation of curcumin silica-lipid hybrid microparticles

According to the method of Tan et al. (22), the microparticles was prepared using a two-step process: the production of silica-stabilised emulsion and drying. Curcumin nanoemulsion was prepared according to the method shown in Preparation of curcumin nanoemulsion. The silica was added into o/w nanoemulsion according different silica-to-lipid ratio by stirring constantly. The silica-stabilised emulsion was then vacuum-dried (DZF-6090, Jinghong, Shanghai, China) under a vacuum pressure of 100 Pa at 50 °C to form solid powder. The drying process was lasted 4h. Different silica-to-lipid mass ratios (1:1, 1:2 and 1:3) were chosen to explore the effect of different additions on the formulation.

Morphological analysis

Transmission Electron Microscopy (TEM)

The morphology characteristics of curcumin nanoemulsion were studied by transmission electron microscopy (TEM) (JEM-2100, JEOL, Tokyo, Japan). The diluted nanoemulsion sample was dropped onto carbon-coated grids and then negatively stained with 2 % (m/V) phosphotungstic acid. Subsequently, the excess liquid was wiped dry by filter paper and the sample was dried at room temperature.

Scanning Electron Microscope (SEM)

The shape and surface characteristics of Aerosil 380 and curcumin silica-lipid hybrid microparticles was explored by scanning electron microscope (SEM) (Ultra Plus, Zeiss, Germany). The powder sample was fixed on a slide using double-sided adhesive tape and the characterization was obtained by a secondary electron detector.

Measurements of particle size and PDI

The average/redispersed particle sizes and polydispersity index (PDI) of the formulations were investigated by dynamic light scattering (DLS) technique (Zetasizer Nano ZS 90, Malvern, UK) at a fixed scattering angle of 90° and room temperature. Before measurement,
0.05 g curcumin nanoemulsion or silica-lipid hybrid microparticles were dispersed in 10 g Milli-Q H₂O. For the microparticles, the aqueous dispersion was centrifuged (TGL-16, Xiangyi, Changsha, China) at 633×g for 10 min in order to precipitate the silica microparticles. Each sample was measured three times.

**Loading content of curcumin**

The loading content of curcumin in nanoemulsion and microparticles were measured by UV/Vis spectrophotometer (755B, Jinghua, Shanghai, China) at the wavelength of 425 nm. 0.1 g nanoemulsion or microparticles was mixed with 9.9 g ethanol to extract the curcumin in the formulations. The curcumin ethanol extractions should be diluted 100 times and centrifuged at 5595×g for 10 min before measurement. Curcumin loading was calculated by the following formula:

\[
\text{Curcumin loading} = \frac{\text{The amount of curcumin}}{\text{The total amount of nanoemulsion or microparticles}} \times 100 /2/ 
\]

**Fourier transform infrared spectroscopy (FT-IR)**

Infrared spectra of curcumin, blank silica-lipid hybrid microparticles, curcumin silica-lipid hybrid microparticles, and the physical mixture of curcumin and blank microparticles (curcumin content was the same as microparticles) were investigated by FT-IR spectrophotometer (Nicolet 6700, Thermo Scientific, USA). The samples were mixed separately with potassium bromide (KBr) and grinded into fine powder. The mixture was processed into a tablet by electric tablet press (DY-30, Keqi, Shanghai, China) under a force of 25 MPa for 10s, and then analyzed at the range of 4000-400 cm⁻¹.

**X-Ray Diffraction (XRD)**

XRD was characterized by X-ray diffraction (D8 Discover, Bruker Corporation, Germany) with CuKα radiation at 30 mA and 40 kV. The diffraction pattern of Aerosil 380, curcumin silica-lipid hybrid microparticles, and the physical mixture of curcumin and Aerosil 380 were
measured over a 2θ range of 5-60°. The scanning speed was 0.15 s/step with a step size of 0.02°.

**In vitro antioxidant activity**

**DPPH eliminating ability**

100 μL various concentrations of curcumin nanoemulsion and silica-lipid hybrid microparticles in ethanol (100 μL of ethanol as blank control group) were added to 3.9 mL DPPH ethanol solution, and then mixed evenly. The reaction was performed at ambient temperature in the dark. Absorbance of the mixture at 517 nm was measured after 30 min of reaction by UV/VIS spectrophotometer. The antioxidant activity was calculated as the following formula:

\[
\text{DPPH scavenging} = \left( 1 - \frac{A_{\text{sample}}}{A_{\text{blank}}} \right) \cdot 100
\]

**Anti-lipid peroxidation effects**

Different concentrations of curcumin nanoemulsion and silica-lipid hybrid microparticles in Milli-Q water were prepared. 0.5 mL of the sample was mixed with 1 mL of 1 % (m/V) soybean phospholipid solution (0.5 mL of Milli-Q H2O as blank control group), 1 mL of 0.2 M (pH=7.4) PBS and 1 mL of 5 mM FeCl2 in the tubes. The mixtures were incubated at 37 °C for 60 min in dark place. Afterwards, 3 mL of TBA-TCA-HCl (contained 0.92 M TCA, 0.025 M TBA and 0.58 M HCl) were added to the mixtures to stop the reaction. The tubes were placed at 95 °C for 15 min then cooled with ice bath. The supernatant was collected after centrifugation at 685xg for 10 min and the absorbance was measured at 532 nm. TBARS inhibition rate is calculated as follows:

\[
\text{TBARS inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \cdot 100
\]

**In vitro simulation of digestion study**

A two-step experiment was performed to simulate the gastric and small intestinal
digestion of curcumin nanoemulsion and silica-lipid hybrid microparticles. Unformulated curcumin was suspended in 0.5 % (m/V) carboxymethylcellulose sodium (CMC-Na) solution as a control group (26). The experiment was referred to the methods described by Huang et al. (27) with small modification.

Simulated gastric digestion: simulated gastric fluid (SGF) was prepared by adding 7 mL HCl (36.5 %) and 2 g NaCl into 1 L Milli-Q water, then adjusted the pH to 1.2 with 1.0 M HCl. 10 mL of nanoemulsion diluent, microparticles aqueous dispersion and curcumin CMC-Na suspension contained the 1 mg/mL of curcumin were mixed with 10 mL of SGF, respectively. The mixture was incubated at 37 °C for 2 h with agitation at 100 rpm after 32 mg of pepsin added into it.

Simulated intestinal digestion: the component of simulated intestinal fluid (SIF) is 0.05 M potassium dihydrogen phosphate solution. Curcumin encapsulated in silica nanoparticle stabilized Pickering emulsion during storage and simulated digestion. The pH of gastric phase was adjusted to 7.0 after simulated gastric digestion. Later, 10 mL of SIF contained 47.6 mg of pancreatin and 51.6 mg of bile extract was added to the mixture and incubated at 37 °C. At this point, the pH-stat titration was utilized to maintain the pH of digestive juice at 7.0 by adding 0.1 M NaOH dropwise. The volume of NaOH was recorded at different time points during 2 h. The percent of free acids released was calculated as the following equation from Ahmed et al. (11):

\[
\text{FFA Released} = \frac{V_{\text{NaOH}} \times C_{\text{NaOH}} \times MW_{\text{Liquid}}}{2 \times W_{\text{Liquid}}} \times 100
\]

Where \( V_{\text{NaOH}} \) is the total volume of NaOH (mL) used for neutralizing FFA released, \( C_{\text{NaOH}} \) is the concentration of NaOH in the burette (0.1M), \( MW_{\text{Liquid}} \) is the molecular weight of the oil in the formula (g/mol), \( W_{\text{Liquid}} \) is the total weight of lipid in the digestive juice.

The particle size of the supernatant of the mixture was measured after each phase.

**In vitro** bioaccessibility: The bioaccessibility of curcumin was measured after simulated digestion. The digestive solution was centrifuged at 5595 \( \times \) g for 10 min and separated into two parts: one was the supernatant containing the mixed micelles, another was the opaque
sediment phase. The amount of curcumin in the supernatant was determined and the bioaccessibility was calculated as following formula:

\[
\text{Bioaccessibility} = \frac{\text{Curcumin in the supernatant}}{\text{The total amount of curcumin}} \cdot 100 /6/
\]

**Storage stability**

Curcumin nanoemulsion and silica-lipid hybrid microparticles were stored at 4 °C, 25 °C and 40 °C (in dark) and 25 °C under daylight for 6 weeks, respectively. Curcumin content was measured every week during the storage (Curcumin ethanol solution was used as a control). Meanwhile, the dispersed particle size and status of microparticles were inspected.

**Cell toxicity evaluation**

Cell culture: L929 murine fibroblast was chosen for cell studies. Cell line was purchased from the type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM medium supplemented with 10 % FBS and 1 % penicillin-streptomycin at 37 °C in a 5 % CO₂ incubator.

Assessment of cell viability: cells were plated in 96-well plates (100 μL/well) at a density of 1·10⁴ cells/mL and cultured for 12 h (blank group was only given an equal volume of cell culture medium, no cells). The curcumin nanoemulsion, blank nanoemulsion, silica-lipid hybrid microparticles and blank particles was diluted to various concentrations (4, 8, 12, 20, 30, 45, 60, 80, 100 and 150 μg/mL) with culture medium and then added to 96-well plates (100 μL/well). Control and blank group were given 100 μL cell culture medium. The media were removed and the cells were washed twice with phosphate buffer solution (PBS). Removed the medium and washed the cells with PBS after 24h treatment. Then, 200 μL CCK-8 solution (10 % in culture medium) was added to the wells for further 1.5 h incubation, the optical density (OD) was recorded by a microplate reader (Thermo, USA) at 450 nm. The cell viability was calculated as follows:

\[
\text{Cell viability} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Control}} - \text{OD}_{\text{Blank}})} \cdot 100 /7/
\]
Statistical analysis

All the experiments were presented in triplicate. Values were expressed as mean and standard deviation (mean±SD). The results were analyzed by ANOVA and t-test. Data was considered as significant for p values < 0.05.

RESULTS AND DISCUSSION

Analysis of Orthogonal experiment

Orthogonal experiment is a method to analyze the correlations of variables at different levels by an orthogonal table and statistical analysis, which can be used for the optimization of multiple formulas (28, 29). The stability of o/w emulsion is closely related to many factors such as the preparation conditions and formulation composition. An orthogonal experiment was designed to optimize the formulation of curcumin nanoemulsion by exploring the effects of preparation temperature and emulsifier concentration on ESI of emulsion. The results and analysis of orthogonal experiment are shown in Table 2. The sequence of effect on ESI grade to evaluate the preparation was: A (temperature) > D (PC 60) > E (PVP K30) > C (Tween 60) > B (Tween 80), which indicated that the temperature had significant effect on the stability of the formula and Tween 80 had little influence on the ESI of the o/w emulsion. Comprehensively considered the performance of each level, the optimum preparation conditions were A4B4C3D2E1. Based on the results, the optimal components of the nanoemulsion were ODO (10 %), curcumin (0.3 %), Tween 80 (5 %), Tween 60 (4 %), PC 60 (3 %), PVP K30 (0.6 %) and Milli-Q H2O (77.1 %), the preparation temperature was 70 °C.

Curcumin silica-lipid hybrid microparticles formation

Microparticles with different silica-to-lipid mass ratios (1:1, 1:2 and 1:3) were prepared by vacuum drying. The sample with 1:3 silica-to-lipid mass ratio appeared as a thick, oily mixture after vacuum drying and there was no powder product formed. The sample with middle silica level could be manifested as yellow agglomerated mass with poor powder mobility. Compared to the previous two formula, the microparticles with 1:1 silica-to-lipid mass ratio formed dry
and free flowing powder which was up to the standard.

**Physicochemical characterization**

The curcumin content, particle size (redispersed particle size) and PDI of curcumin nanoemulsion and microparticles are tabulated in Table 3. The particle size of nanoemulsion was further analyzed by TEM. The formation of nanoemulsion is due to the hydrophobic tails of emulsifier molecules attach to hydrophobic core formed by the oil phase while the hydrophilic head protrude into aqueous phase, which form small spheroid particles dispersed in the aqueous phase. The spherical structure of nanoemulsion droplets could be observed in Fig. 1a. The result of dynamic light scattering test showed the particle size of nanoemulsion was (40.53±2.37) nm, which was substantiated by the diameter of particles in the TEM. PDI of nanoemulsion and microparticles was (0.164±0.018) and (0.264±0.018), respectively. The particle size and PDI of nanoemulsion were significantly smaller than microparticles with (177.18±3.26) nm. This could be ascribed to the oil droplet interfaces absorbed tiny silica particles, leading to an increase in particle size of the redispersed microparticles (30). The SEM images of Aerosil 380 and microparticles are illustrated in Fig. 1. In previous study (31), the Aerosil 380 was the aggregates of 7 nm sized primary particles, which demonstrated a porous matrix internal structure with pore sizes ranging from 25 to 100 nm. The SEM images (Fig. 1b) showed a loose, porous and near-spherical shape which gave the particles high specific surface area and strong adsorption ability. Nevertheless, the SEM of microparticles (Fig. 1c) lost these features and performed a rough surface, which demonstrated microparticles formulation is based on the oil phase was adsorbed onto the porous silica matrices. The results were consistent with the ideas of Simovic et al (21) in which droplets are uniformly distributed and absorbed onto the surface of silica instead of forming a continuous film. In other studies, Tan et al. (22) proposed that there could be redistribution and self-assembly of the Aerosil 380 particles from the continuous phase to the droplet surface and the inner wick during dehydration process. The curcumin content of nanoemulsion and microparticles were (0.30±0.016) % and (0.67±0.019) %, respectively, indicating that curcumin
loading was increased after solidification.

**XRD**

Physical state of curcumin in the silica-lipid hybrid microparticles was investigated by XRD analysis. The XRD spectrum of curcumin, Aerosil 380, physical mixture of curcumin and Aerosil 380, curcurmin microparticles are shown in Fig. 2a. Physical mixture of curcumin and Aerosil 380 displayed several sharp diffraction peaks in the diffractogram indicated that curcumin existed in crystalline form. In contrast, the XRD spectrum of microparticles was similar to that of Aerosil 380 particles and there was no characteristic peak observed. The results suggested that curcumin performed as a XRD-amorphous state in the microparticles.

**FTIR**

FTIR was chosen to investigate the interactions between curcumin and excipients in the solid state. The FTIR spectra of curcumin, blank- silica-lipid hybrid microparticles, physical mixture of curcumin and blank- silica-lipid hybrid microparticles, curcumin silica-lipid hybrid microparticles are shown in Fig. 2b. A characteristic peak observed at 3508 cm\(^{-1}\) in the spectra of curcumin powder corresponded to the O-H stretching vibration, which was different than the broad peak of amorphous curcumin in the study of Li et al (32). This phenomenon suggested a different molecular environment of hydroxyl groups between crystalline curcumin and amorphous curcumin. The peak at 1627 cm\(^{-1}\) was relative to the C=O group and the band at 1509 cm\(^{-1}\) represented the presence of C=C group. Meanwhile, these characteristic peak still existed in the spectra of physical mixture, indicating no interaction occurred between curcumin and blank-microparticles. However, the characteristic peaks were disappeared in curcumin microparticles and the spectra was similar to that of blank- microparticles. Therefore, the results forecasted that curcumin was incorporated in Aerosil 380 and remained a dissolved state in the microparticles (33,34).
In vitro antioxidant activity

DPPH free radical scavenging assay

DPPH is a stable radical which has been extensively applied in antioxidant analytic techniques. Generally, DPPH signals decrease when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H (35). In this study, the DPPH assays was used to explore the free radical scavenging abilities of curcumin nanoemulsion and microparticles. According to the results in Fig. 3a, the nanoemulsion and microparticles showed a higher DPPH scavenging abilities than curcumin ethanol solution. The DPPH scavenging was 87.98 % (nanoemulsion) and 87.06 % (microparticles) compared to the control of 81.17% when the concentration came to 1 mg/mL. It can be postulated that the nanoparticles protected curcumin from degradation and thus increased the antioxidant ability. In addition, the radical scavenging ability of microparticles was slightly lower than that of nanoemulsion when the concentration was less than 1 mg/mL. Explanation from Sari et al (36) was the encapsulation of silica not only reduce the degradation of curcumin but may also preserve the antioxidant activity. Hence, this result indicated that the free radical scavenging abilities of curcumin were not affected by the encapsulation of nanocarriers.

Anti-lipid peroxidation

Lipid peroxidation is often relevant to a few biological damages, particularly the biological membranes of brain, liver (37). Generally, malondialdehyde (MDA) is one of the major products of lipid peroxidation, whose levels can be spectrophotometrically determined by forming a complex with the TBA (2-thiobarbituric acid) called TBA reactive substances (TBARS). The TBARS inhibition of curcumin nanoemulsion, silica-lipid hybrid microparticles and curcumin ethanol solution are shown in Fig. 3b. It was observed that the anti-lipid peroxidation capacities of nanoemulsion and microparticles were increased in a dose dependent manner, and the inhibition rate was over 50 % at the concentration of 100 μg/mL. However, the TBARS inhibition of microparticles were slightly lower compared with curcumin
ethanol solution. It can be explained as the influence of the slow release of curcumin from Aerosil 380.

In vitro simulation of digestion study

A two-step experiment was used to explore the simulated digestion process of curcumin nanoemulsion and silica-lipid hybrid microparticles. The particle sizes of digestive juice were measured after each digestion phase by DLS technique. The changes in particle size were shown in Fig. 4a. Nanoemulsion and microparticles before digestion had mean particle size of (40.53±2.37) nm and (177.18±3.26) nm, respectively. After gastric digestion process, there was not much change in particle sizes of the two samples. This result illustrated that the structures of the two samples kept well and the oil droplets were not destroyed during the gastric digestion process. However, after 2h of intestinal digestion, the particle sizes of nanoemulsion and microparticles increased significantly to (100.05±2.32) nm and (821.95±4.72) nm, respectively. The augmentation of particle sizes suggested that droplets was destabilized in the process of simulated intestinal digestion. Previous research showed that bile salts can take the place of surfactant molecules from oil-water interface, facilitate the binding of pancreatic enzymes to the oil-water interfacial layer and promote the lipid digestion, leading to the destabilization of emulsion (38, 39). And the complex reactions that occurred in intestine result in the formation of mix micelles which could transport the encapsulated components to the surfaces of the enterocytes and enhanced the absorption of nutrition (40).

During the intestinal digestion, oil droplets will be broken down into free fatty acids and monoglycerides thus the bioactive molecules could be released (41). In this study, the degree of lipid digestion from the nanoemulsion and microparticles was measured by instilling 0.1M NaOH into digestive juice to neutralize free fatty acids. As shown in Fig. 4b, there was a rapid increase in the rate of free fatty acids released from nanoemulsion and microparticles in the first 30min, followed by a more gradual increase from 30 to 120 min. However, there were some differences in the rate and extent of digestion between nanoemulsion and microparticles. The initial rate of nanoemulsion was faster than that of microparticles, which can be attributed to the difference on droplet size. A number of studies have found that the
release rate of free fatty acids increases as the lipid droplets size decrease (42). The droplets could have more contact with lipase molecules on account of the large specific surface area of small droplets (43). Moreover, the final amount of free fatty acids released of microparticles was lower than nanoemulsion, which indicated that the microparticles can inhibit the release of oil droplets. The possible reason was that the oil-water interfaces were adsorbed by silica particles which led to the inhibition of oil release (30).

Bioassessibility is adjudged as the fraction of the active ingredient which is solubilized from the food during simulate gastrointestinal digestion, so it is considered as an estimable method of oral bioavailability (44). The bioaccessibility of the samples were measured at the end of simulated intestinal digestion. As shown in Fig. 4c, there was a significantly increase in bioaccessibility of nanoemulsion and microparticles compared to curcumin-sodium carboxymethyl cellulose (Cur-CMC-Na) suspension, indicating that nanocarriers can improve the bioavailability of curcumin. However, it was obvious from the results that the bioaccessibility of nanoemulsion was higher than that of microparticles. This phenomenon was similar to the results of free fatty acids released that the bioaccessibility increased as the rate of free fatty acids released increased. The difference in curcumin bioaccessibility between nanoemulsion and microparticles may due to two reasons. Lower amount of free fatty acids released caused more undigested lipid present, which could reserve the curcumin in it. Furthermore, there were not enough mixed micelles existent to dissolve the curcumin (45).

Storage stability

Stability is an important factor in examining the quality of the system. In this study, the retention ratios of curcumin in nanoemulsion and silica-lipid hybrid microparticles was measured during 6 weeks’ storage at different temperatures to explore the chemical stability of the two samples. Fig. 5a was shown that the curcumin retention ratios of nanoemulsion did not change significantly at 4 °C and 25 °C, and had slight changes at 40 °C storage temperature. On the whole, the nanoemulsion maintained good chemical stability during the 6 weeks’ storage. The same result was also displayed on microparticles (Fig. 5b). However, the manifestation of the
samples stored in light condition were different from those in dark condition (Fig. 5c). During 6 weeks’ storage, the curcumin retention ratios of nanoemulsion declined to 31.20% which suggested the formulation was unstable under light conditions and should be stored in a light-free condition. The retention ratio of microparticles also appeared a decrease and remained at near 73.23%. Even so, it was still clearly higher than that of nanoemulsion. This observation was confirmed that solid preparations had a better protection for curcumin when exposed to light. The possible reason was that the curcumin was encapsulated in the pores of Aerosil 380 which could reduce the influence of light on curcumin (45). Meanwhile, the dispersed particle size and status of curcumin microparticles were inspected during the storage, the results were shown in Table 3. The dispersed particle size and appearance have not changed significantly, which indicating a good physical stability.

**Cell toxicity evaluation**

Viability of L929 cells was measured by CCK-8 assay. Results were shown in Fig.6. The preliminary cell studies demonstrated that the blank carriers does not have negative effect on the cell viability, which indicated high safety of the excipients used in formula. Curcumin nanocarriers deterred the cell viability in a dose dependent manner. The cell viability was over 80% when the concentration of nanocarriers was less than 45 μg/mL. And the viability dropped to less than 50% when L929 cells were treated with 150 μg/mL nanocarriers. The results indicated that curcumin at a certain concentration had evident cytotoxicity. In addition, the same concentration of curcumin microparticles performed less cytotoxic than nanoemulsion when the concentration was more than 30 μg/mL, which probably due to the incomplete release of curcumin in the solid preparation.

**CONCLUSION**

In this study, curcumin-loaded o/w nanoemulsion and curcumin silica-lipid hybrid microparticles were successfully optimized to improve the solubility and bioavailability. Orthogonal experiment was used to obtain an optimum emulsion formula and the
microparticles was prepared on the basis of nanoemulsions by a two-step process which showed good flow properties. XRD analysis suggested that curcumin performed as XRD-amorphous state instead of crystalline state in the microparticles. FTIR experiment showed that curcumin was encapsulated in Aerosil 380 and remained a dissolved state in the microparticles. In vitro anti-oxidative activity experiment confirmed that both two formulations had good capacity for scavenging DPPH free radical and anti-lipid peroxidation. Results from in vitro digestion showed that the products digestion was mainly occurred in intestinal juice on account of the effects of bile salts and pancreatic enzymes. Compared with nanoemulsion, the rate and extent of free fatty acids released were reduced when the lipid was incorporated with Aerosil 380, which was due to the restraint of microparticles on the lipolysis of oil droplets. Moreover, the bioaccessibility of curcumin was significantly higher for nanoemulsion and microparticles in comparison with curcumin CMC-Na suspensions, which indicated that curcumin-lipid complexes could enhance the absorption of curcumin. The two formulations could maintain good chemical stability for 6 weeks at different temperatures in the dark conditions and the microparticles had a better protection for curcumin from degradation under light conditions. The curcumin carriers showed a low cell cytotoxicity when the concentration was less than 45 μg/mL. It can be concluded that the solid preparation has better stability and is a promising approach to improve the bioavailability for curcumin.

FUNDING

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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.
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Q. Xia https://orcid.org/0000-0001-6123-8918

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https://doi.org/10.1016/j.scitotenv.2010.10.021

https://doi.org/10.1246/cl.2012.1334

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<th>Level</th>
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<th>C/%</th>
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<td>5.0</td>
<td>5.0</td>
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</tbody>
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Where A means the temperature (°C) of preparation, and B, C, D, E is the concentration of Tween 80, Tween 60, PC 60, PVP K30 in the formula, respectively.
Table 2. The result of orthogonal experiment

<table>
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<th>B/%</th>
<th>C/%</th>
<th>D/%</th>
<th>E/%</th>
<th>Cur /%</th>
<th>ODO /%</th>
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<th>Score /%</th>
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</tr>
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k_1 = 32.24, k_2 = 36.21, k_3 = 44.22, k_4 = 58.58, R = 26.37
Where A means the temperature (°C) of preparation, and B, C, D, E is the concentration (%) of Tween 80, Tween 60, PC 60, PVP K30 in the formula, respectively.

Table 3. Status, dispersed particle size and PDI of the microparticles

<table>
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<tr>
<th>Temperature /°C</th>
<th>Storage time /week</th>
<th>Status</th>
<th>Dispersed particle size/nm</th>
<th>PDI</th>
</tr>
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<td>Yellow powder</td>
<td>178.02±1.82</td>
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<tr>
<td></td>
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<td>Yellow powder</td>
<td>177.98±2.05</td>
<td>0.259±0.017</td>
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<tr>
<td></td>
<td>6</td>
<td>Yellow powder</td>
<td>178.84±1.27</td>
<td>0.247±0.026</td>
</tr>
<tr>
<td>25</td>
<td>2</td>
<td>Yellow powder</td>
<td>179.42±1.57</td>
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<tr>
<td></td>
<td>4</td>
<td>Yellow powder</td>
<td>179.27±0.09</td>
<td>0.262±0.014</td>
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<tr>
<td></td>
<td>6</td>
<td>Yellow powder</td>
<td>181.49±2.08</td>
<td>0.261±0.021</td>
</tr>
<tr>
<td>40</td>
<td>2</td>
<td>Yellow powder</td>
<td>180.66±2.34</td>
<td>0.265±0.012</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Yellow powder</td>
<td>178.97±3.02</td>
<td>0.263±0.009</td>
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<tr>
<td></td>
<td>6</td>
<td>Yellow powder</td>
<td>179.28±2.05</td>
<td>0.272±0.013</td>
</tr>
</tbody>
</table>
Please note that this is an unedited version of the manuscript that has been accepted for publication. This version will undergo copyediting and typesetting before its final form for publication. We are providing this version as a service to our readers. The published version will differ from this one as a result of linguistic and technical corrections and layout editing.

Fig. 1. a) TEM of curcumin nanoemulsion (Cur-NE), b) SEM of Aerosil 300, and c) curcumin silica-lipid hybrid microparticles (Cur-SLH).

Fig. 2. a) XRD of curcumin, Aerosil 300, physical mixture of curcumin and Aerosil 300, curcumin silica-lipid hybrid microparticles (Cur-SLH). b) FTIR of curcumin, blank silica-lipid hybrid microparticles (Blank-SLH), physical mixture of curcumin and blank SLH, Cur-SLH microparticles (Cur-SLH).
Fig. 3. a) DPPH scavenging activity of curcumin ethanol solution (Cur-EtOH), curcumin nanoemulsion (Cur-NE) and curcumin silica-lipid hybrid microparticles (Cur-SLH). Results are shown as mean±SD (n=3). b) TBARS inhibition of Cur-EtOH, Cur-NE and Cur-SLH. Results are shown as mean±SD (n=3).

Fig. 4. a) Particle sizes of curcumin nanoemulsion (Cur-NE) and curcumin silica-lipid hybrid
microparticles (Cur-SLH) before digestion, in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Results are shown as mean±SD (n=3). *** or ### means significantly differences (p≤0.01). b) FFA cumulative released of Cur-NE and Cur-SLH during the simulated gastric digestion process. Results are shown as mean±SD (n=3). c) Bioaccessibility of Cur-CMC-Na suspension (as a control group), Cur-NE and Cur-SLH after the simulated digestion process. Results are shown as mean±SD (n=3). *** means significantly differences (p≤0.01).

Fig.5. Retention ratios of curcumin in a) curcumin nanoemulsion (Cur-NE) and b) curcumin silica-lipid hybrid microparticles (Cur-SLH) during the storage of 6 weeks at 4 °C, 25 °C and 40 °C in dark. c) Retention ratios of curcumin in curcumin ethanol solution (Cur-EtOH), Cur-NE and Cur-SLH during the storage of 6 weeks at 25 °C under light. Results are shown as mean±SD (n=3).
Fig. 6. Cell viability of curcumin nanoemulsion (Cur-NE), blank nanoemulsion (Cur-NE), curcumin silica-lipid hybrid microparticles (Cur-SLH) and blank silica-lipid hybrid microparticles (Blank-SLH). Results are shown as mean±SD (n=3).