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Determination of oral bioavailability of curcuminoid dispersions and nanoemulsions prepared from *Curcuma longa* Linnaeus

Pei Shan Lu, Baskaran Stephen Inbaraj and Bing Huei Chen*

Abstract

BACKGROUND: Curcuminoid from *Curcuma longa* Linnaeus has been demonstrated to be effective in anti-cancer and anti-inflammation. The objectives of the present study were to prepare curcuminoid dispersion and nanoemulsion from *C. longa* and determine their oral bioavailabilities in rats.

RESULTS: After curcuminoid extraction using 99.5% ethanol, bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC) and curcumin were separated within 10 min by high-performance liquid chromatography using an Eclipse XDB-C18 column (Agilent, Palo Alto, CA, USA) and a gradient mobile phase of 0.1% aqueous formic acid and acetonitrile, with a flow rate of 1 mL min^{-1}, column temperature of 35 °C and detection wavelength of 425 nm. Curcuminoid nanoemulsion at a particle size of 12.1 nm and encapsulation efficiency 98.8% was prepared using lecithin, Tween 80 and water. A pharmacokinetic study in rats revealed that the parameters including T_{max}, C_{max}, $t_{1/2}$ and the area under the curve were higher for curcuminoid nanoemulsions than for curcuminoid dispersion at the same dose employed for gavage administration, whereas, for intravenous injection, an opposite trend was shown. The oral bioavailabilities of BDMC, DMC, curcumin and total curcuminoids in nanoemulsion and dispersion were 34.39 and 4.65%, 39.93 and 5.49%, 47.82 and 9.38%, and 46 and 8.7%, respectively.

CONCLUSION: The results of the present study demonstrate a higher oral bioavailability after incorporation of curcuminoid into nanoemulsion, facilitating its application as a botanic drug.
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Keywords: curcuminoid nanoemulsion; *Curcuma longa* Linnaeus; animal model; oral bioavailability; HPLC analysis

INTRODUCTION

Curcuma longa Linnaeus, an important medicinal herb reported to possess well-established biological activities including anti-cancer, anti-inflammation and anti-Alzheimer's disease,[1–3] has been widely used by consumers in Asian countries such as India and China. Most importantly, clinical trial experiments have demonstrated that, even with a high daily dose of 8 g of curcumin, the major functional component in *C. longa*, no adverse effect was observed for patients.[4] Thus, curcumin possesses a great potential to be developed as a botanic drug with respect to its safety and possible therapeutic efficiency in chronic diseases. However, several issues remain, including its poor bioavailability, fast metabolism and systemic elimination *in vivo* after oral administration.

Among the various functional components in *C. longa*, curcuminoid represents the most important class because it constitutes approximately 2–6% and is composed of curcumin (80%), demethoxycurcumin (DMC) (18%) and bisdemethoxycurcumin (BDMC) (2%).[4] Being insoluble in aqueous solution and susceptible to degradation under alkaline and light conditions, the application of curcuminoids in the food or drug industry is limited. Therefore, identifying an appropriate way of enhancing aqueous solubility and stability of curcuminoids both *in vitro* and *in vivo* is of great importance. Numerous studies have shown that, by encapsulating curcuminoid into a microemulsion or nanoemulsion system, both the aqueous solubility and stability of curcuminoids can be greatly enhanced.[5,6] However, most studies deal with the preparation of microemulsion or nanoemulsion from a curcumin standard and not curcuminoid extract from *C. longa*.

Theoretically, pharmacokinetic experiments are mandatory for new drug development prior to clinical trials because they can describe the phenomenon of absorption, distribution, metabolism and excretion *in vivo*. Drug absorption is a process of drug intake to systemic circulation, with lipophilic drugs being absorbed by passive diffusion from a high to low concentration, whereas hydrophilic drugs are taken up by active transportation through a carrier allowing penetration into the cell membrane for systemic circulation.[7] Thus, factors affecting drug absorption often include size, dose, formulation of drug intake, encapsulation efficiency, administration route and release rate, etc. On the other hand, the determination of drug concentration in the blood is a direct way of assessing drug concentration *in vivo*, with intravenous (i.v.) injection being regarded as an administration route for complete absorption and often being used as a control treatment in

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pharmacokinetic studies. Obviously, through determination of bioavailability, the rate and extent to which a bioactive ingredient such as curcumin is absorbed and becomes available at the site of action *in vivo* can be clarified. Several studies have shown that curcumin nanoemulsion/nanoparticles could provide better pharmacokinetic parameters [C_{max}, T_{max}, $t_{1/2}$ and area under the curve (AUC)] and higher bioavailability than curcumin dispersion.[8–13] However, in most published studies, the oral bioavailability of curcumin nanoparticles remains relatively low, probably as a result of an inadequate encapsulation efficiency and large size (>100 nm).[8–10] Thus, there is a need to reduce the curcumin nanoparticle size down to < 100 nm with a high encapsulation efficiency to raise oral bioavailability. The objectives of the present study were to extract curcuminoids from *C. longa* and prepare curcuminoid nanoemulsion for enhancement of encapsulation efficiency and to decrease nanoparticle size so that the oral bioavailability of curcuminoid can be raised. In addition, the oral bioavailabilities of curcumin, DMC, BDMC and total curcuminoids in rats were determined by clarifying various pharmacokinetics parameters, including T_{max}, C_{max}, $t_{1/2}$ and AUC using the WinNonlin pharmacokinetic software system (Pharsight, St Louis, MO, USA).

MATERIALS AND METHODS

Materials

A total of 5 kg of *C. longa* was provided by Hua-Lian Agricultural Experiment Station (Hua-Lian County, Taiwan), which was subjected to hot-air drying and vacuum package for storage at −20 °C before use. Curcumin standard was procured from Enzo Life Sciences Co. (New York, NY, USA). DMC and BDMC standards, as well as internal standard methyl red, were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Food-grade Tween 80 and lecithin were obtained from Yu-Pa Enterprise Co. (Taipei, Taiwan) and Cheng-Fang Co. (Taipei, Taiwan), respectively. Both heparin and potassium dihydrogen phosphate were obtained from Sigma-Aldrich Co. High-performance liquid chromatography (HPLC)-grade solvents including methanol and acetonitrile were obtained from Lab-Scan Co. (Gliwice, Poland). Formic acid was also obtained from Sigma-Aldrich Co. Deionized water was made using a Milli-Q water purification system from Millipore Co. (Bedford, MA, USA). An Eclipse XDB-C18 column (inner diameter 150 x 4.6 mm, particle size 5 μm) was obtained from Agilent (Palo Alto, CA, USA).

Extraction of curcuminoids

A method based on a previous study by Zhan *et al.*[14] was modified to extract curcuminoid from *C. longa*. Initially 10 g of dried *C. longa* powder was mixed with 100 mL of ethanol in a flask, after which the mixture was sonicated for 30 min, followed by shaking in a low-temperature circulation water bath shaker (Firstek B402L; Li-Chen Instrument Co., Taoyuan, Taiwan) at room temperature for 1 h and centrifuging at 10 000 × *g* at 4 °C for 10 min in high-speed centrifuge (Sorvall RC5C; DuPont Co., Wilmington, DE, USA). Next, the supernatant was collected and the bottom layer was repeatedly extracted until colorless. All of the supernatants were combined and filtered through a filter paper (6 μm). Then, the filtrate was evaporated to dryness under vacuum in a rotary evaporator (N-1; Eyela Co., Tokyo, Japan), dissolved in 10 mL of anhydrous ethanol and filtered through a 0.22-μm membrane filter to obtain curcuminoid extract for subsequent experiment.

HPLC analysis of curcuminoids in *C. longa*

A method based on that reported by Chang and Chen[5] was used to separate curcuminoids in *C. longa* extract. An Agilent 1100 series HPLC system comprising a G1379A degasser, a G1311A quaternary pump, a G1316A column temperature controller and a G1315B photodiode-array detector was used. In addition, a 6130 quadrupole mass spectrometer with multimode ion source (electrospray ionization and atmospheric pressure chemical ionization) was used for curcuminoid identification. After various studies, an Eclipse XDB-C18 column and a mobile phase of 0.1% formic acid solution (A) and acetonitrile (B) with the following gradient condition was developed: 60% A and 40% B in the beginning, decreased to 36% A at 7 min, maintained for 3 min, decreased to 10% A at 15 min, and returned to original ratio at 17 min. A total of three curcuminoids including curcumin, BDMC and DMC, as well as internal standard methyl red, were separated within 10 min with a column temperature of 35 °C, a flow rate of 1 mL min^{-1} and a detection wavelength of 425 nm. The purity of each peak was automatically determined by an Agilent G2180A Spectral Evaluation Software System, which is based on degree of spectra overlapping of each peak. The various curcuminoids in *C. longa* extract were identified by comparing retention time, absorption spectra and mass spectra of unknown peaks with reference standards. The mass spectra of curcumin, BDMC and DMC were determined using the same approach as that described in a previous study.[5]

Method validation

For quantitation, the internal standard methyl red was dissolved in acetonitrile to obtain a concentration of 20 μg mL^{-1}. Seven concentrations of curcumin (0.03, 0.1, 0.2, 1, 5, 10 and 20 μg mL^{-1}) were prepared in acetonitrile, whereas seven concentrations of BDMC or DMC (0.03, 0.05, 0.1, 0.3, 0.5, 1 and 2 μg mL^{-1}) were also prepared in acetonitrile. Then, each curcuminoid standard was mixed with methyl red and injected into HPLC three times, and the standard curves were prepared by plotting the concentration ratio (standard *versus* internal standard) against area ratio (standard *versus* internal standard). The linear regression equation and correlation coefficient (*R*) of each standard curve was automatically obtained using SigmaPlot, version 10 (Systat Software Inc., Chicago, IL, USA).

Both intra-day variability (repeatability) and inter-day variability (intermediate precision) were determined based on a method described by the International Conference on Harmonization.[15] The former was carried out by injecting sample extract three times each in the morning, afternoon and evening on the same day, whereas the latter was performed by injecting sample extract once each in the morning, afternoon and evening on the first, second and third day. Both the limit of detection (LOD) and limit of quantitation (LOQ) were also determined based on a method described by the International Conference on Harmonization.[15] Three concentrations of 0.02, 0.1 and 0.2 μg mL^{-1} were each prepared for curcumin, BDMC or DMC. Each concentration was injected into HPLC three times and the standard curves were prepared by plotting concentration against peak area, with the slope (s) and intercept (σ) being obtained from the linear regression equation of each standard curve. Then both LOD and LOQ were calculated as:

$$LOD = 3.3 \times (\sigma/s)$$

$$LOQ = 10 \times (\sigma/s)$$

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For recovery determination, two volumes (100 and 500 μL) of curcumin, BDMC and DMC standards (1000 μg mL^{-1} each) were each added to 1 g of *C. longa* powder for extraction. After HPLC analysis, the recovery of each curcuminoid was calculated based on the relative ratio of the amount of curcuminoid standard after HPLC to that before HPLC.

Preparation of curcuminoid dispersion and nanoemulsion

Initially 5 mL of curcuminoid extract was collected in a vial and ethanol was evaporated under nitrogen. Then, 0.75 g of lecithin and 1 mL of Tween 80 was added and mixed homogeneously, which was followed by adding 5.3 mL of deionized water and mixing thoroughly. Then, the solution was sonicated for 30 min at 400 W and 40 kHz (Delta® DC400H; Chuan-Hua Co., Taipei, Taiwan) to obtain curcuminoid nanoemulsion, which was stored at 4 °C before use. For preparation of curcuminoid dispersion, the extract was evaporated and dissolved in a mixture of dimethyl sulfoxide and deionized water (1:99, v/v).

Nanoemulsion characterization

A portion of curcuminoid nanoemulsion (0.1 mL) was diluted 50 times with 25 mmol L^{-1} phosphoric acid dihydrogen potassium buffer solution (pH 5.5). After mixing thoroughly, the solution was filtered through a 0.22-μm membrane filter and poured into a polystyrene colorimetric tube for dynamic light scattering analysis (Brookhaven Co., New York, NY, USA). Also, the size and shape of curcuminoid nanoemulsion was determined by transmission electron microscopy (TEM), which is based on diffraction contrast imaging to produce a bright or dark field. Initially, a portion of curcuminoid nanoemulsion was diluted 50 times with deionized water, after which a 20-μL sample was dropped on a copper grid for 30 s, followed by the removal of extra sample with a glass filter paper, and adding 20 μL of phosphotungstic acid (2%) for 30 s for negative staining. After removal of the excessive solution with a glass filter paper, the sample was placed in an oven for drying for TEM analysis using a JEM-1400 TEM instrument (JEOL, Tokyo, Japan) at 120 kVA. For zeta potential determination, the sample preparation method was the same as for dynamic light scattering (DLS) analysis. Then, the sample was placed in a zeta potential analyzer (Horiba Co., Kyoto, Japan), with the potential range from −200 to +200 mV and a temperature of 25 °C. For the encapsulation study, a portion of curcuminoid nanoemulsion was poured into a centrifuged tube containing a dialysis membrane [molecular weight (MW) cut-off 3000 Da] with deionized water in the lower layer. Free curcuminoid could penetrate into the membrane to disperse in the aqueous layer, whereas the encapsulated curcuminoid remained on the membrane surface. Then, the free curcuminoid in the aqueous layer was analyzed by HPLC for determination of encapsulation efficiency.[5] For a stability study, the curcuminoid nanoemulsion was stored at 4 and 25 °C for 6 months, during which a portion of sample was collected every 2 weeks for determination of particle size distribution by DLS.

Animal experiment

Male Sprague–Dawley rats at 4 weeks of age and a body weight (BW) of 70–100 g were obtained from BioLASCO Laboratory Animal Center (Taipei, Taiwan). These animals were housed in the Fu Jen University Animal Center, which was maintained at an ambient temperature of 22 ± 2 °C and a relative humidity of 55 ± 10% for 12 h (07.00–19.00 h). All rats were fed with a laboratory rodent diet 5001 (LabDiet, St Louis, MO, USA) *ad libitum*. This animal study was approved by the Fu Jen University animal subjects review committee and strict regulations on human care for laboratory animals were adopted. After 4 weeks, all rats with a body weight in the range 280–300 g were ready for the experiments and were prohibited from feeding for 12 h before sacrifice.

For a total of 36 rats, the first three groups with six rats each were administered orally (gavage), whereas the remaining three groups with six rats each were injected i.v. Based on several pre-experiments, a dose of 0.1 g kg^{-1} BW was chosen for oral administration of curcuminoid nanoemulsion and dispersion to two groups separately, whereas a lower dose of 0.05 g kg^{-1} BW curcuminoid nanoemulsion was administered to one group orally. After oral administration for 2, 5, 10 and 30 min, and also 1, 2, 4, 8, 24, 48 and 72 h, 0.5 mL of blood sample was collected from the tail vein and poured into a heparin-rinsed tube. Then, the blood samples were transferred to ice bath and stood for 30 min, followed by centrifugation at 10 000 × *g* for 10 min (4 °C). The supernatant (plasma) was collected for subsequent experiments. For i.v. administration, curcuminoid nanoemulsion and dispersion were injected through temporal vein to two groups separately at a dose of 0.02 g kg^{-1} BW, whereas a lower curcuminoid nanoemulsion dose of 0.01 g kg^{-1} BW was also injected in one group. After i.v. injection for 2, 5, 10 and 30 min, as well as 1, 2, 4, 8, 24, 48 and 72 h, 0.5 mL of blood sample was collected from the tail vein and poured into a heparin-rinsed tube for subsequent experiments, as described above.

HPLC analysis of curcuminoids in rat plasma

Plasma sample was vortex-mixed with 0.5 mL of ethanol containing the internal standard methyl red (20 μg mL^{-1}) for protein precipitation, followed by centrifugation to obtain supernatant. The residue was repeatedly extracted with ethanol until colorless. All the supernatants were combined, evaporated under N_2, dissolved in 1 mL acetonitrile and filtered through a 0.22-μm membrane filter for HPLC analysis using the same mobile phase as described above. For quantitation, the standard curves of BDMC, DMC and curcumin were also prepared using the same method as described before. Both LOD and LOQ were determined by preparing three concentrations of curcumin (0.01, 0.02 and 0.1 μg mL^{-1}), as well as of BDMC and DMC (0.01, 0.03 and 0.1 μg mL^{-1} each). After injection into HPLC three times for each concentration, the standard curves were prepared by plotting concentration against area to obtain slope (s) and intercept (σ). Then both LOD and LOQ were calculated using the same formula as described before.

For matrix effect determination, seven concentrations of 0.03, 0.1, 0.2, 1, 5, 10 and 20 μg mL^{-1} for curcumin and seven concentrations of 0.03, 0.05, 0.1, 0.3, 0.5, 1 and 2 μg mL^{-1} each for BDMC and DMC were mixed with 20 μg mL^{-1} of internal standard methyl red and injected separately into HPLC to obtain a standard calibration curve (SCC) for quantitation. Similarly, the matrix matched calibration curve (MCC) was obtained by mixing the same seven concentrations of curcumin, BDMC and DMC as shown above for SCC with blank plasma extract containing 20 μg mL^{-1} internal standard methyl red, followed by injection into HPLC for curcuminoid analysis. Then, by substituting the slopes obtained from SCC and MCC, the matrix effect can be calculated based on the formula:

$$\text{Matrix effect } (\%) = \frac{\text{MCC slope} - \text{SCC slope}}{\text{SCC slope}} \times 100$$

For recovery determination, two volumes (10 and 20 μL) of BDMC, DMC and curcumin (1000 μg mL^{-1} each) were added to

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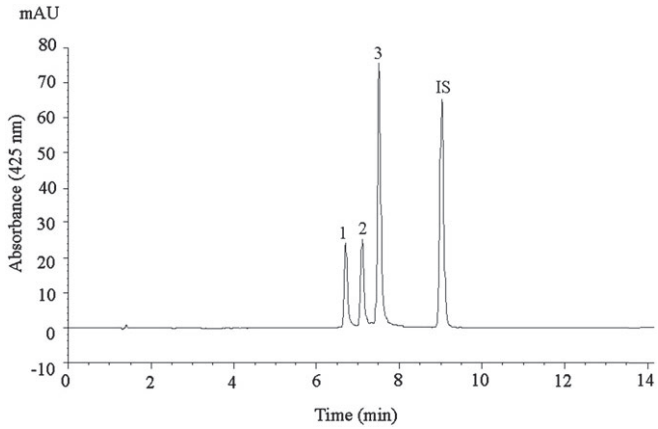

Figure 1. HPLC chromatogram of curcuminoids extracted from *C. longa*. Column, C18; binary mobile phase, 0.1% formic acid solution and acetonitrile; flow rate, 1 mL min^{-1}; detection wavelength, 450 nm; peak 1, bisdemethoxycurcumin; peak 2, demethoxycurcumin; peak 3, curcumin.

plasma samples separately for extraction and HPLC analysis. Then, the recovery of each curcuminoid was obtained based on the formula described above.

Pharmacokinetic parameters analysis

All of the the data with respect to curcuminoids in plasma were expressed as the mean ± SD. The various pharmacokinetic parameters including AUC (area under the curve), C_{max} (maximum concentration in plasma), T_{max} (time to maximum concentration in plasma) and $t_{1/2}$ (half-life) were determined automatically with WinNonlin, version 5.2.1 (Pharsight). Then, the absolute bioavailability of curcumin, BDMC, DMC and total curcuminoids were determined using the formula:

$$\text{Absolute bioavailability } (\%) = \frac{(\text{AUC}_\infty)\, P_0/D_0P_0\,(\text{dose by gavage})}{(\text{AUC}_\infty)\,\text{i.v.}/D_0\text{i.v.}\ (\text{dose by i.v.})} \times 100$$

Where $(AUC_{0-\infty})P_0$ is AUC obtained by gavage; $(AUC_{0-\infty})$i.v. is AUC obtained by i.v.; D_0P_0 is dose by gavage; and D_0i.v. is dose by i.v.

Statistical analysis

All of the curcuminoid data for quantitation were carried out in triplicate by HPLC analysis. The data are expressed as the mean ± SD for quantitation of curcumin, BDMC and DMC from the linear regression equation of each standard curve as obtained using Excel 2007 (Microsoft Corp. Redmond, WA, USA).

RESULTS AND DISCUSSION

HPLC analysis of curcuminoids in *C. longa*

In many earlier studies, the various curcuminoids in *C. longa* were often separated by thin-layer chromatography with silica gel 60 as adsorbent and various combinations of solvents, such as ethyl acetate, chloroform, acetic acid, methanol and hexane, as mobile phase.[16,17] However, both the resolution and purity of BDMC, DMC and curcumin remained inadequate. Thus, in recent years, many HPLC methods were developed to separate BDMC, DMC and curcumin by employing a C18 column and a combination of water and acetonitrile or methanol containing acid as mobile phase.[14,18] After various studies, a gradient mobile phase composed of acetonitrile and 0.1% formic acid in water as described in the Materials and methods was developed to separate BDMC, DMC and curcumin within 10 min (Fig. 1). Table 1 shows retention time (t_R), retention factor (k), separation factor (α), peak purity and mass spectra of various curcuminoids in *C. longa*, with the t_R in the range 6.63–7.45 min, k in the range 4.82–5.54, α in the range 1.07–1.24 and peak purity in the range 93.2–98.4%. Both k and α values implied that a proper solvent strength of mobile phase and a suitable selectivity of mobile phase to sample components were attained, as indicated by an adequate resolution of BDMC, DMC and curcumin in Fig. 1.

The quality control data of curcuminoids in *C. longa* by HPLC analysis is shown in Table 2. The relative standard deviation (RSD) of BDMC, DMC and curcumin for the intra-day variability was 1.66%, 0.64% and 0.68%, respectively, whereas the inter-day variability was 1.16%, 0.62% and 0.59%, respectively, demonstrating a high repeatability and reproducibility (precision) of our HPLC method. Similarly, a high accuracy of this method was achieved, as shown by a high recovery of 101.8%, 97.4% and 93.1% for BDMC, DMC and curcumin, respectively. Both LOD and LOQ were determined to be 0.003 and 0.009 ppm, respectively, for BDMC and curcumin, as well as 0.01 and 0.03 ppm for DMC. For quantitation, the linear regression equations of BDMC, DMC and curcumin were $y = 3.6365x + 0.0042$, $y = 3.1289x + 0.0045$ and $y = 4.0743x - 0.0354$, respectively, with the correlation coefficient (R) being higher than 0.99. Of the various curcuminoids, curcumin was present in the largest amount (11.43 g kg^{-1}), followed by DMC (4.53 g kg^{-1}) and BDMC (3.52 g kg^{-1}) (Table 2). It is worth noting that the original curcuminoid content in *C. longa* shown in Table 2 is the same as that for the intra-day variability data, as based on

Table 1. Retention time (t_R), retention factor (k), separation factor (α), purity and MS spectra data of curcuminoids extracted from *C. longa*

Peak number	Compound	t_R (min)	Retention factor (k)	Separation factor (α)	Peak purity (%)	[M-H]$^-$ (m/z)
1	BDMC[a]	6.63	4.82	1.09 (1,2)[d]	93.2	306
2	DMC[b]	7.04	5.18	1.07 (2,3)[d]	97.9	336
3	curcumin	7.45	5.54	1.24 (3,4)[d]	98.4	367
4	Methyl red[c]	8.96			99.9	

[a] Bisdemethoxycurcumin.
[b] Demethoxycurcumin.
[c] Internal standard.
[d] Numbers in parentheses represent values between two neighboring peaks.

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Table 2. Quality control data of curcuminoids extracted from *Curcuma longa* by HPLC-DAD analysis

Peak number	Curcuminoids	LOD[c] (ppm)	LOQ[d] (ppm)	Recovery (%)	Intra-day variability[e] Mean ± SD (g kg^{-1})	Intra-day variability[e] RSD (%)	Inter-day variability[e] Mean ± SD (g kg^{-1})	Inter-day variability[e] RSD (%)	Content (g kg^{-1})[e]
1	BDMC[a]	0.003	0.009	101.8	3.52 ± 0.06	1.66	3.47 ± 0.04	1.16	3.52 ± 0.06
2	DMC[b]	0.01	0.03	97.4	4.53 ± 0.03	0.64	4.51 ± 0.03	0.62	4.53 ± 0.03
3	Curcumin	0.003	0.009	93.1	11.43 ± 0.08	0.68	11.37 ± 0.07	0.59	11.43 ± 0.08

[a] Bisdemethoxycurcumin.
[b] Demethoxycurcumin.
[c] Limit of detection.
[d] Limit of quantitation.
[e] Mean ± SD of triplicate analyses.

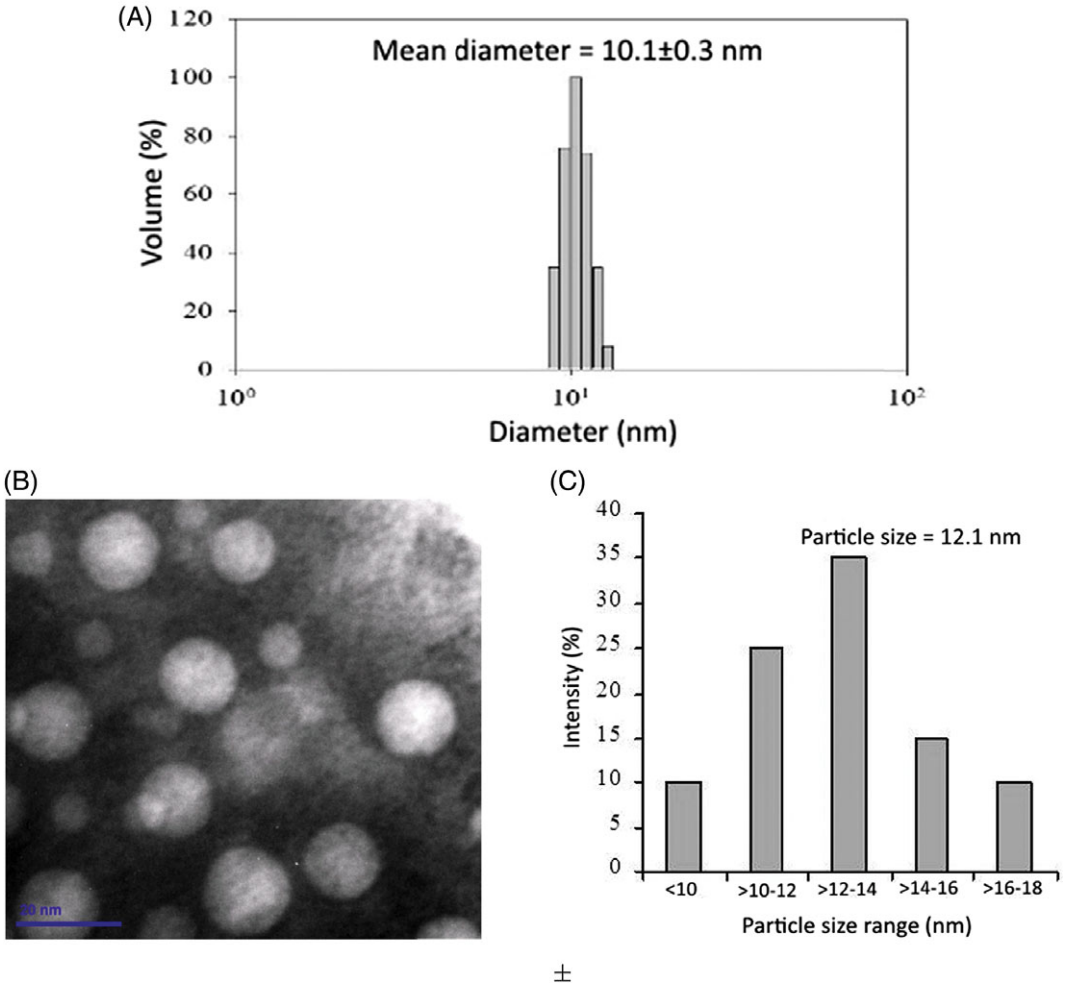

Figure 2. DLS particle size distributio n (A) and TEM image (B) of curcuminoids nanoemulsion along with particle size distribution obtained directly from the TEM image (C).

triplicate HPLC analyses of curcuminoids carried out in the morning, afternoon and evening on the same day. In several previous studies, the recovery data of curcumin or curcuminoids as reported by Sun *et al.*[19], Lechtenberg *et al.*[20] and Jadhav *et al.*[21] are in agreement with ours. Similarly, total curcuminoids were reported to be in the range 5.66–40.36 g kg^{-1} in *C. longa* cultivated in different areas of China, whereas the curcumin level was in the range 4.18–22 g kg^{-1}.[18] This finding is also similar to the present study, as shown in Table 2.

Characteristics of curcuminoid nanoemulsion

Figure 2 shows the particle size distribution of curcuminoid nanoemulsion as determined by the DLS method, and an average particle size of 10.1 ± 0.3 nm was found. Likewise, the average particle size of curcuminoid nanoemulsion with spherical shape as determined by TEM was 12.1 nm (Fig. 2). Compared to many published reports, the particle size of curcuminoid nanoemulsion prepared in the present study was much smaller, which can be attributed to a difference in formulation. For example, several

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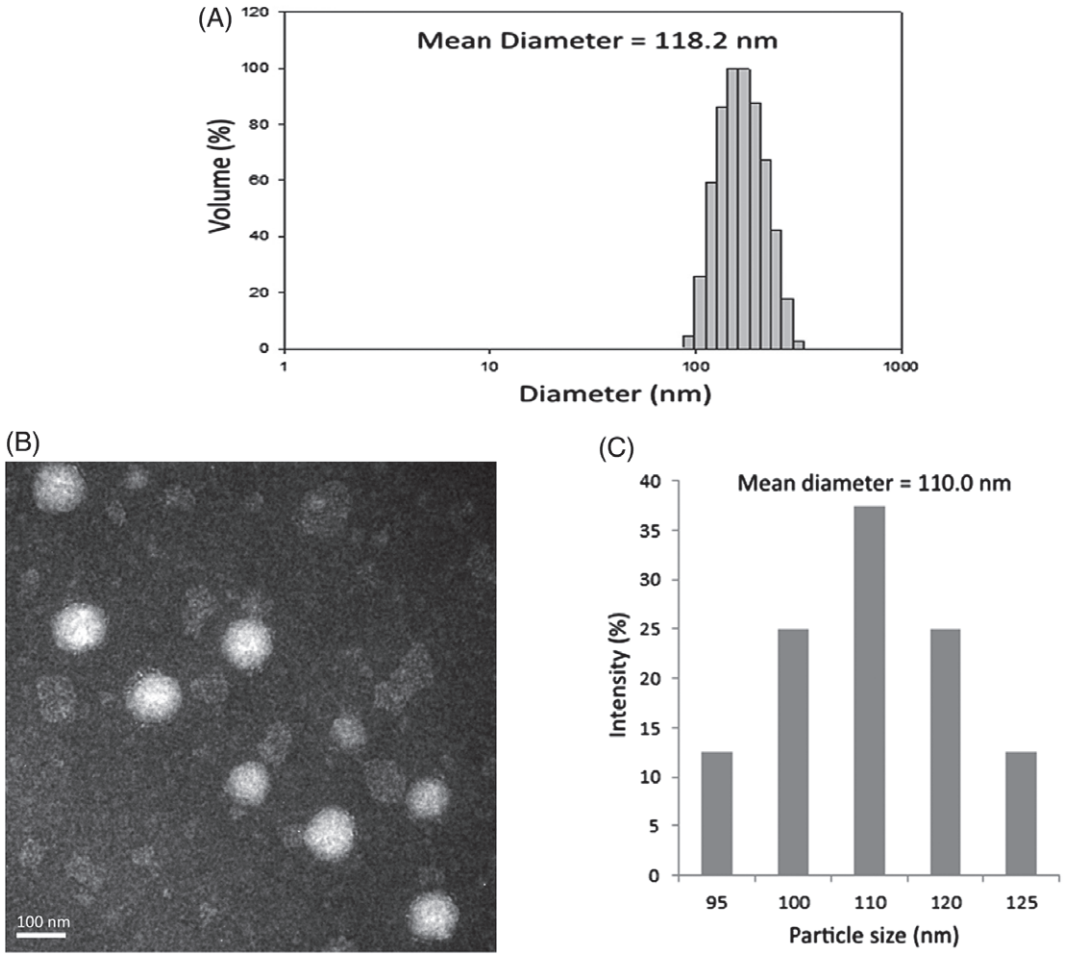

Figure 3. DLS particle size distribution (A) and TEM image (B) of curcuminoids dispersion along with particle size distribution obtained directly from the TEM image (C).

studies used polymers such as polylactic-co-glycolic acid (PLGA) or polyethylene glycol as the major component to prepare curcuminoid nanoemulsion for encapsulation and enhancement of both stability and oral bioavailability. However, the size of curcuminoid nanoemulsion is often > 100 nm.[8,13] Conversely, in the present study, no polymer was used for preparation of curcuminoid nanoemulsion, and thus the size could be reduced substantially. In addition, the zeta potential and encapsulation efficiency of our curcuminoid nanoemulsion were determined to be −56.2 mV and 98.8%, respectively. On the other hand, curcuminoid dispersion showed a particle size of 118.2 and 110 nm, as determined by DLS and TEM, respectively (Fig. 3), with the zeta potential and encapsulation efficiency remaining undetected. More importantly, a high stability of our curcuminoid nanoemulsion was observed over a storage period of 6 months at 4 and 25 °C, as indicated by a mean particle size of 12.4 ± 0.5 nm and 16.7 ± 0.6 nm, respectively, after prolonged storage. This stability can be attributed to high negative zeta potential of our curcuminoid nanoemulsion, as it has been demonstrated that a high positive (>30) or negative (< −30) zeta potential can contribute greatly to the stability of microemulsion or nanoemulsion because of the presence of highly-charged surfaces that resist droplet aggregation.[22]

In several previous studies, Wang *et al.*[6] developed a nanoparticulate formulation of curcumin encapsulated in stearic acid-g-chitosan oligosaccharide (CSO-SA) polymeric micelles and reported the mean particle size and encapsulation efficiency to be 114.7 nm and 29.9%, respectively. Although the curcumin-loaded CSO-SA micelles are effective with respect to inhibiting subpopulations of $CD44^+/CD24^-$ cells (putative colorectal cancer stem cell markers) both *in vitro* and *in vivo*, both the zeta potential (18.5 mV) and encapsulation efficiency (29.9%) remained low. In another study, Zhang *et al.*[23] developed a folate-modified self-microemulsifying drug delivery system composed of 57.5% Cremophor®EL, 32.5% Transcutol®HP, 10% Capryol™ 90, and a small amount of folate-polyethylene glycol-cholesteryl hemisuccinate for encapsulation of curcumin and colon cancer cell targeting. This system showed a pronounced toxicity towards colon cancer cells. However, the mean particle size and zeta potential were shown to be 31.3 nm and −6.56 mV, respectively, revealing a low stability of this system. Similarly, Wang *et al.*[24] developed a methoxy poly(ethylene glycol)-poly(Caprolactone) (MPEG-PCL) micelle loaded with curcumin and doxorubicin (Cur-Dox/MPEG-PCL) and reported that the Cur-Dox/MPEG-PCL was effective in anti-tumor (lung cancer) both *in vitro* and *in vivo*, with the mean particle size and encapsulation efficiency being 38.4 nm and 98.7%, respectively. Nevertheless, the zeta potential was only −0.269 mV, indicating a low stability of this micelle. More recently, Liu *et al.*[25] prepared an amphiphilic block copolymer, *N*-*t*-butoxycarbonyl-phenylalanine terminated monomethoxy-poly(ethylene glycol)-b-poly(ε-caprolactone), for encapsulation of

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Table 3. Method validation parameters for HPLC analysis of curcuminoids in rat plasma

Curcuminoid	Concentration range (μg mL^{-1})[c]	Linear regression: Standard calibration curve (SCC)[d]	Linear regression: Matrix-matched calibration curve (MCC)[d]	LOD (ppm)[e]	LOQ (ppm)[e]	Recovery (%)[f]	Matrix effect (%)[g]
BDMC[a]	0.03–2	$y = 3.6365x + 0.0042$	$y = 3.0638x + 0.0031$	0.009	0.027	93.82	2.1
DMC[b]	0.03–2	$y = 3.1289x + 0.0045$	$y = 3.559x - 0.0053$	0.001	0.003	92.78	2.1
Curcumin	0.03–20	$y = 4.0743x - 0.0354$	$y = 3.6472x - 0.0336$	0.002	0.006	103.7	−11

[a] Bisdemethoxycurcumin.
[b] Demethoxycurcumin.
[c] Each curcuminoid concentration was prepared in acetonitrile containing 20 μg mL^{-1} internal standard methyl red for obtaining standard calibration curve, whereas, for matrix matched calibration, each curcuminoid concentration was spiked into blank plasma containing 20 μg mL^{-1} internal standard methyl red.
[d] Both SCC and MCC were obtained by triplicate HPLC analyses and plotting concentration ratio (standard *versus* internal standard) against area ratio (standard *versus* internal standard).
[e] Limit of detection and limit of quantitation was obtained based on triplicate HPLC analyses of three concentrations (0.01, 0.03 and 0.1 μg mL^{-1} for BDMC and DMC, as well as 0.01, 0.02 and 0.1 μg mL^{-1} for curcumin) spiked in blank plasma as shown for the MCC method.
[f] Recovery determination was based on spiking two volumes of 10 and 20 μL from 1000 μg mL^{-1} of BDMC, DMC and curcumin standards separately into blank plasma and calculating the relative ratio of amount of each curcuminoid standard after HPLC to that before HPLC.
[g] Matrix effect was calculated by substituting slopes from SCC and MCC in the formula, matrix effect (%) = [(MCC slope − SCC slope)/SCC slope] × 100.

dimethoxycurcumin (DMC) in the micelle, with an average particle size and encapsulation efficiency of 17.9 nm and 97.22%, respectively. However, the zeta potential and stability of the DMC-loaded micelle during storage remained undetected. As shown above, most of the reported nanocurcuminoids possess either large particle size, low zeta potential or low encapsulation efficiency. By contrast, the curcuminoid nanoemulsion prepared in the present study showed a relatively smaller particle size (12.1 nm), higher zeta potential (−56.2 mV) and encapsulation efficiency (98.8%), implying its potential to exhibit high bioavailability as a result of a high surface-to-volume ratio, stability and encapsulation efficiency.

Method validation of HPLC analysis of curcuminoids in rat plasma

Table 3 shows the method validation parameters for HPLC analysis of curcuminoids in rat plasma. Blank plasma extract was mixed with various concentrations of curcuminoids standards, with the final concentrations of curcumin being 0.03, 0.1, 0.2, 1, 5, 10 and 20 μg mL^{-1}, and both BDMC and DMC being 0.03, 0.05, 0.1, 0.3, 0.5, 1 and 2 μg mL^{-1}. After HPLC analysis, the standard curve (matrix matched calibration curve) of each curcuminoid in plasma was prepared by plotting concentration against area, and the linear regression equations of curcumin, BDMC and DMC were $y = 3.6472x - 0.0336$, $y = 3.0638x + 0.0031$ and $y = 3.559x - 0.0053$, respectively, with R being higher than 0.99. For determination of both LOD and LOQ, three concentrations of 0.01, 0.02 and 0.1 μg mL^{-1} were prepared for curcumin, whereas 0.01, 0.03 and 0.1 μg mL^{-1} were prepared for both BDMC and DMC. Following the same approach as described in the Materials and methods, the LOD of curcumin, DMC and BDMC was determined to be 0.002, 0.001 and 0.009 μg mL^{-1}, respectively, whereas the LOQ was 0.006, 0.003 and 0.027 μg mL^{-1}, respectively. The recoveries of BDMC, DMC and curcumin were 93.82%, 92.78% and 103.7%, respectively, with the RSD being 1.72%, 0.96% and 2.35%.

For determination of matrix effect, both standard curves and matrix matched calibration curves were prepared as described above. After calculation of the slope difference using the formula described in the Materials and methods, the matrix effect of BDMC, DMC and curcumin was determined to be 2.1%, 2.1% and −11%, respectively, revealing a minimal matrix effect of curcuminoids in rat plasma during extraction.

Pharmacokinetic study of curcuminoids in rat plasma

Figure 4(A, B) shows the HPLC chromatograms of curcuminoids in rat plasma collected at 2 min, 2 h and 72 h after gavage administration at 0.1 g kg^{-1} BW and i.v. administration at 0.05 g kg^{-1} BW, respectively. All the three curcuminoids, BDMC, DMC and curcumin, as well as internal standard (methyl red), were adequately separated within 10 min by HPLC. By comparison, both DMC and curcumin were present at a higher level by i.v. administration for 2 min compared to that by gavage administration at the same time point. However, after gavage administration for 72 h, only a small amount of DMC and curcumin was detected, whereas, after i.v. administration at the same time point, BDMC, DMC and curcumin were undetected.

Figure 5 shows the concentration–time profiles of BDMC, DMC and curcumin in rat plasma following gavage and i.v. administration of curcuminoid nanoemulsion. At the same dose of 0.1 g kg^{-1} BW, the nanoemulsion showed a higher concentration of BDMC, DMC and curcumin compared to those in the dispersion at each time point. Also, after gavage administration of nanoemulsion at 0.1 g kg^{-1} BW, the time to reach maximum concentration (T_{max}) of BDMC, DMC and curcumin was 60, 120 and 120 min, respectively, whereas the maximum concentration (C_{max}) was 0.03, 0.04 and 0.14 μg mL^{-1} (Table 4). In addition, curcumin showed the highest level of AUC (246.8 min μg mL^{-1}), followed by DMC (44.52 min μg mL^{-1}) and BDMC (7.76 min μg mL^{-1}). The same trend was observed for $t_{1/2}$ (Table 4). Comparatively, a high-dose nanoemulsion for gavage administration (0.1 g kg^{-1} BW) can increase the C_{max} of BDMC, DMC and curcumin substantially in rat plasma, all of which were two- to four-fold higher compared to those in low-dose nanoemulsion (0.05 g kg^{-1} BW). Also, the T_{max} of BDMC, DMC and curcumin in nanoemulsion (0.1 g kg^{-1} BW) was longer than that in dispersion at the same dose and nanoemulsion at a lower dose (0.05 g kg^{-1} BW). Additionally, the blood circulation time was prolonged pronouncedly, as indicated by a longer $t_{1/2}$ of the nanoemulsion at 0.1 g kg^{-1} BW. Similarly, the highest AUC levels of BDMC, DMC and curcumin were found for nanoemulsion at the same dose by gavage administration,

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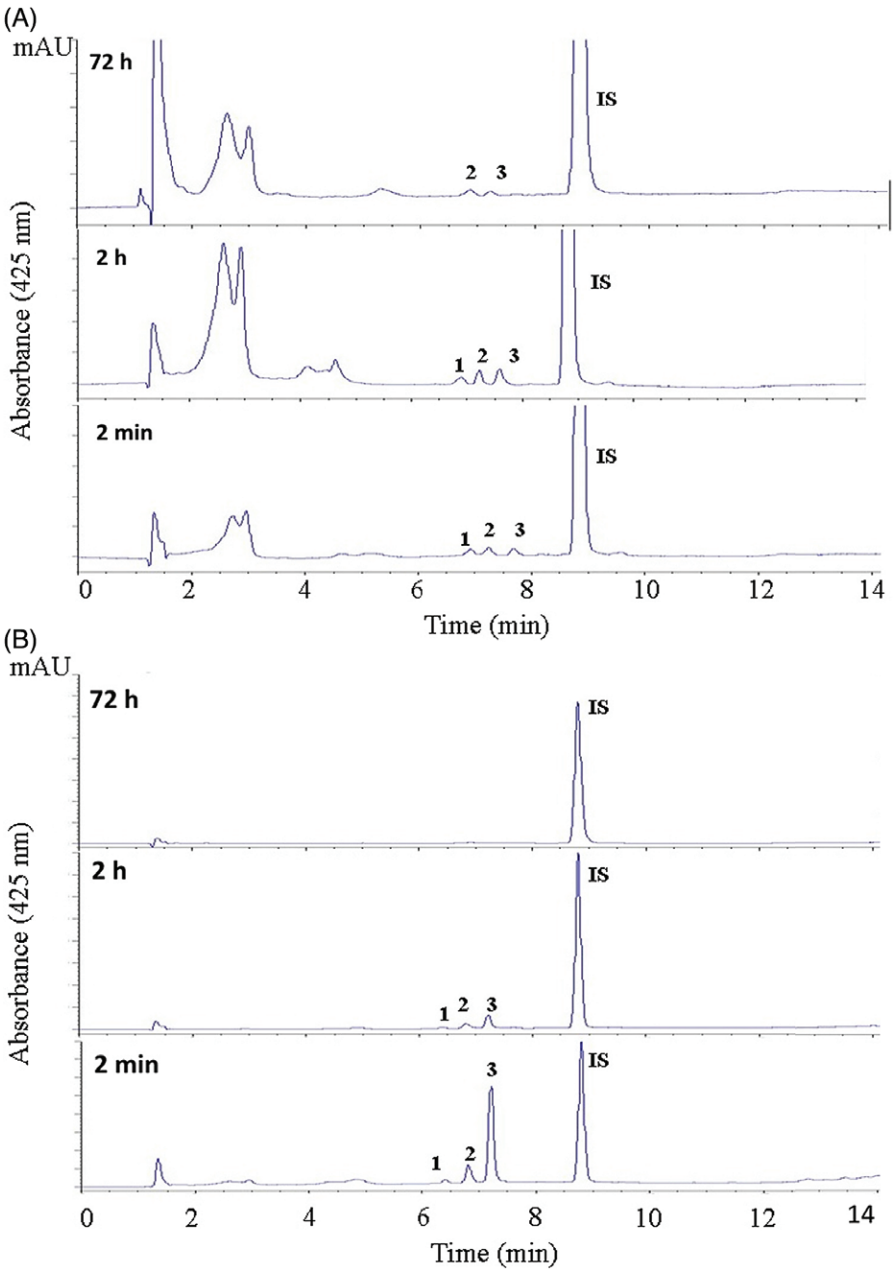

Figure 4. HPLC chromatogram of curcuminoids in rat plasma collected at 2 min, 2 h and 72 h after gavage (A) and i.v. (B) administration. Curcuminoid dose, 0.1 g kg^{-1}; peak 1, bisdemethoxycurcumin; peak 2, demethoxycurcumin; peak 3, curcumin; IS, internal standard methyl red.

followed by dispersion at the same dose and nanoemulsion at 0.05 g kg^{-1} BW. By contrast, the C_{max} of BDMC, DMC and curcumin in dispersion after i.v. administration (0.02 g kg^{-1} BW) was higher than that in nanoemulsion at both doses of 0.02 and 0.01 g kg^{-1} BW. Both $t_{1/2}$ and AUC of BDMC, DMC and curcumin showed a similar trend. It may be assumed that, after i.v. injection, curcuminoid nanoemulsion can be transported into tissues or organs faster from blood circulation than curcuminoid dispersion, probably because of presence of large particle size of the latter. Thus, at the same time point and dose, the C_{max}, AUC and $t_{1/2}$ of BDMC, DMC and curcumin in nanoemulsion were lower compared to those in dispersion.

However, for oral administration, curcuminoid nanoemulsion was absorbed faster in the intestinal tract for subsequent circulation into blood as a result of the presence of a small particle size. Conversely, curcuminoid dispersion needed to be metabolized or degraded into small molecules prior to absorption in the intestinal tract for blood circulation. Therefore, at the same time point and dose, the nanoemulsion treatment showed a higher C_{max} and AUC, as well as longer $t_{1/2}$, than the dispersion treatment for BDMC, DMC and curcumin. Also, for gavage administration, a higher C_{max} and AUC, as well as a longer T_{max} and $t_{1/2}$ of BDMC, DMC and curcumin in nanoemulsion, was shown with a high dose (0.1 g kg^{-1} BW) compared to a low dose (0.05 g kg^{-1} BW). By contrast, for i.v. injection, a lower C_{max} and AUC of BDMC, DMC and curcumin in nanoemulsion was observed at a high dose (0.02 g kg^{-1} BW) than at a low dose (0.01 g kg^{-1} BW), probably because of oversaturation and fast circulation into organs such as the liver.

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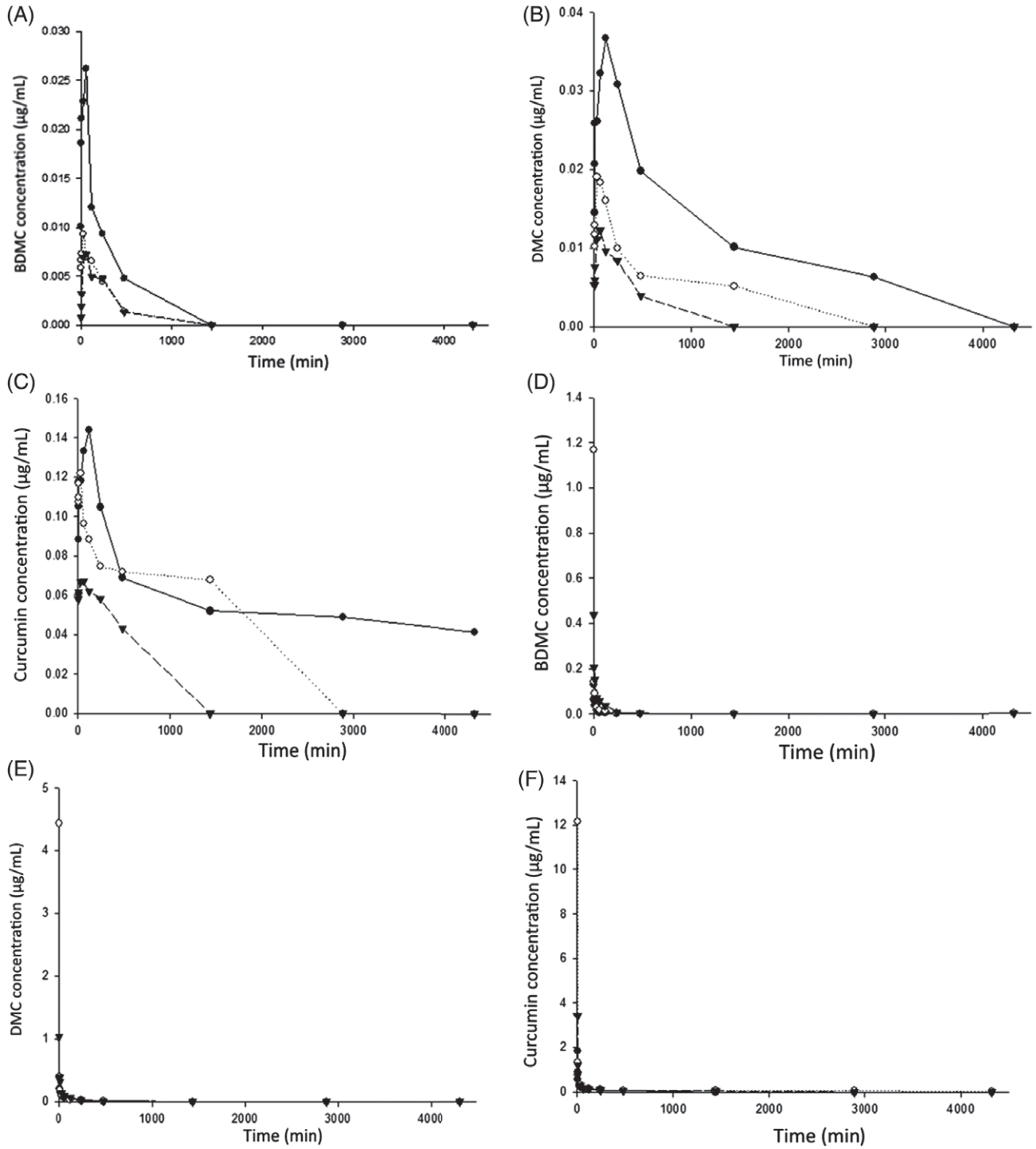

Figure 5. Concentration-time profiles for BDMC (A, D), DMC (B, E) and curcumin (C, F) in rat plasma following gavage (A–C) and i.v. (D–F) administration of curcuminoids nanoemulsion. The number of replicates is six per time point ($n = 6$). The data label (○) in all the profiles represent gavage and i.v. administration of curcuminoids dispersion at 0.1 and 0.02 g kg^{-1} BW, respectively, whereas the data labels (●) and (▼) denote high- and low-dose administration of curcuminoids nanoemulsion by gavage (0.1 and 0.05 g kg^{-1} BW) and i.v. (0.02 and 0.01 g kg^{-1} BW).

Interestingly, the $t_{1/2}$ showed the same trend as for gavage administration. Because the nanoemulsion treatment showed a longer $t_{1/2}$ by gavage administration than by i.v. injection, this nanoemulsion should be more suitable for oral administration. Conversely, the $t_{1/2}$ of the dispersion by i.v. injection was higher than that by gavage administration, indicating that the dispersion should be more appropriate for i.v. injection. Taken together, at the same dose of 0.1 g kg^{-1} BW, the oral bioavailability of BDMC, DMC and curcumin was determined to be 34.39%, 39.93% and 47.82%, respectively, for the nanoemulsion treatment, and 4.65%, 5.49% and 9.38%, respectively, for the dispersion treatment (Table 4). However, for the nanoemulsion treatment at a low dose of 0.05 g kg^{-1} BW, the oral bioavailability of BDMC, DMC and curcumin was 3.42%, 3.5% and 5.69%, respectively, being only

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Table 4. Pharmacokinetic parameters of BDMC, DMC, curcumin and total curcuminoid as well as oral bioavailability in rats following gavage and i.v. administration

	Gavage administration[a]											
	Nanoemulsion (0.1 g kg^{-1} BW[b])				Dispersion (0.1 g kg^{-1} BW)				Nanoemulsion (0.05 g kg^{-1} BW)			
Parameters	BDMC[c]	DMC[d]	Curcumin	Total curcuminoid	BDMC	DMC	Curcumin	Total curcuminoid	BDMC	DMC	Curcumin	Total curcuminoid
T_{max} (min)[e]	60	120	120	120	30	30	30	30	60	60	60	60
C_{max} (μg/mL)[f]	0.03	0.04	0.14	0.19	0.01	0.02	0.12	0.15	0.01	0.01	0.07	0.09
$T_{1/2}$(min)[g]	231	1386	2310	2310	210	770	1733	1733	182	630	990	866
AUC(min μg/mL)[h]	7.76	44.52	246.80	299.08	2.91	14.92	155.31	173.14	2.68	5.72	47.85	56.24
	i.v. administration[a]											
	Nanoemulsion (0.02 g kg^{-1} BW[b])				Dispersion (0.02 g kg^{-1} BW)				Nanoemulsion (0.01 g kg^{-1} BW)			
Parameters	BDMC[c]	DMC[d]	Curcumin	Total curcuminoid	BDMC	DMC	Curcumin	Total curcuminoid	BDMC	DMC	Curcumin	Total curcuminoid
T_{max} (min)[e]	–	–	–	–	–	–	–	–	–	–	–	–
C_{max} (μg/mL)[f]	0.13	0.38	1.83	2.35	1.17	4.43	12.15	17.75	0.44	1.03	3.43	4.9
$T_{1/2}$(min)[g]	103	173	365	126	147	408	6930	866	75	83	347	301
AUC(min μg/mL)[h]	4.51	22.30	103.22	130.03	12.53	54.36	331.09	397.5	15.69	32.66	168.14	216.46
Oral bioavailabilty (%)[i]	34.39	39.93	47.82	46	4.65	5.49	9.38	8.7	3.42	3.50	5.69	5.2

[a] Mean data based on analyses of blood samples collected from six rats (n=6).
[b] Body weight.
[c] Bisdemethoxycurcumin.
[d] Demethoxycurcumin.
[e] Time to maximum concentration in plasma.
[f] Maximum concentration in plasma.
[g] Time to half concentration in plasma.
[h] Area under the concentration-time curve.
[i] Absolute bioavailability as calculated based on a formula shown in Materials and methods.

slightly lower than that for the dispersion treatment at a high dose of 0.1 g kg^{-1} BW. This finding further demonstrated that the nanoemulsion dose could be reduced by one half to attain a similar oral bioavailability of the dispersion at the same dose, which can be a result of an enhanced stability of the nanoemulsion during digestion and absorption *in vivo*. The pharmacokinetic parameters of total curcuminoids in rats following gavage and i.v. administration are also shown in Table 4. It was observed that the higher the dose, the higher the C_{max} of total curcuminoids in rat plasma by gavage administration (Fig. 6). Similar to curcumin, BDMC and DMC, the oral bioavailability of total curcuminoids for both nanoemulsions at a high dose (0.1 g kg^{-1} BW) and low dose (0.05 g kg^{-1} BW) and dispersion at 0.1 g kg^{-1} BW was 46%, 5.2% and 8.7%, respectively.

In several previous studies Shaikh *et al.*[8] conducted a pharmacokinetic experiment through oral administration of various formulations and doses of curcumins to rats: 0.1 g kg^{-1} BW curcumin nanoparticles encapsulated with PLGA, suspension composed of 0.25 g kg^{-1} BW curcumin standard and 0.01 g kg^{-1} BW piperine, as well as 0.25 g kg^{-1} BW curcumin suspension. The results showed that the C_{max} was 260.5 ± 26.4, 121.2 ± 23.1 and 90.3 ± 15.3 ng mL^{-1}, respectively, whereas the T_{max} was 2, 0.75 and 0.5 h, respectively, implying that curcumin suspension was more susceptible to excretion than nanoparticles. Also, the oral bioavailability of nanocurcumin was 26-fold higher than for curcumin suspension, as indicated by a much higher AUC (3224 ± 329 ng mL^{-1} h) for the former compared to the latter (312 ± 9 ng mL^{-1} h). Similarly, Onoue *et al.*[9] compared the oral bioavailability of crystallized curcumin standard (0.1 g kg^{-1} BW), nanocrystal solid curcumin dispersion (0.02 g kg^{-1} BW), amorphous solid curcumin dispersion (0.02 g kg^{-1} BW) and curcumin nanoemulsion (0.02 g kg^{-1} BW) and reported that the bioavailability was 0.9%, 14.3%, 10.7% and 7.9%, respectively. In addition, both nanocrystal solid curcumin dispersion and amorphous solid curcumin dispersion possessed a relatively higher AUC compared to the other two treatments. Nevertheless, in comparison with the other treatments, curcumin nanoemulsion exhibited a very short $t_{1/2}$ (39 ± 10 min), revealing a possible fast excretion in rats.

In another study, Tsai *et al.*[10] prepared PLGA-encapsulated nanocurcumin with the average size and encapsulation efficiency of 158 ± 10 nm and 46.6 ± 13.5%, respectively. Upon i.v. injecting 0.0025 g kg^{-1} BW of nanocurcumin and 0.01 g kg^{-1} BW of non-nanocurcumin into rats, the mean residence time (MRT) was shown to increase from 11.6 min for the former to 18.8 min for the latter, whereas the value of AUC/dose increased from 3.1 to 4.8, indicating that the residence time of curcumin in rats could be prolonged through preparation of nanocurcumin. After oral and i.v. administration of nanocurcumin at 0.05 and 0.0025 g kg^{-1} BW, respectively, the oral bioavailability was found to be 4.71%. Similarly, Feng *et al.*[11] prepared curcumin loaded PCL-PEG-PCL triblock copolymeric nanoparticles and injected into rats i.v. (0.015 g kg^{-1} BW) and the AUC and MRT of curcumin nanoparticle were both found to be 4.15- and 178.99-fold higher compared to that of the corresponding non-nanocurcumin, respectively. Shargel *et al.*[12] noted that T_{max} can be used as an index of drug absorption rate (i.e. the smaller the T_{max}, the faster the drug absorption). In

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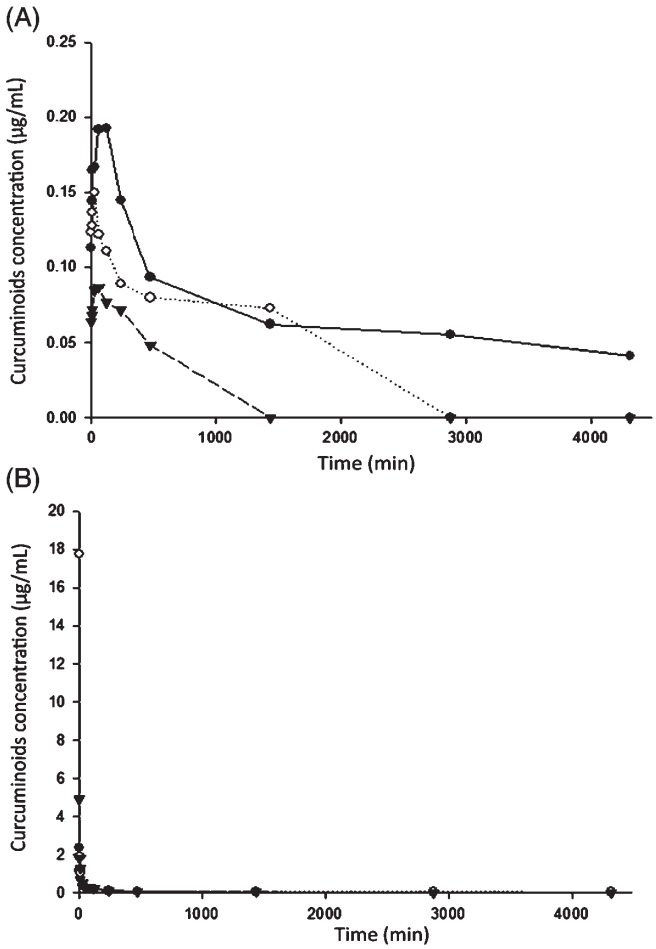

Figure 6. Concentration-time profiles of curcuminoids in rat plasma following gavage (A) and i.v. (B) administration of curcuminoids nanoemulsion. The number of replicates is six per time point ($n = 6$). The data label (○) in both the profiles represent gavage and i.v. administration of curcuminoids dispersion at 0.1 and 0.02 g kg^{-1} BW, respectively, whereas the data labels (●) and (▼) denote high- and low-dose administration of curcuminoids nanoemulsion by gavage (0.1 and 0.05 g kg^{-1} BW) and i.v. (0.02 and 0.01 g kg^{-1} BW).

a later study, Tsai *et al.*[13] compared the effect of high-MW and low-MW nanocurcumin on bioavailability in rats, and the former was shown to have a higher bioavailability because of a shorter T_{max}. The foregoing discussion on reported nanocurcuminoids revealed relatively better pharmacokinetic parameters and a higher bioavailability after incorporation of curcuminoid into nanoformulations compared to its dispersion counterpart.

By comparison, the present study demonstrated a higher oral bioavailability of curcuminoids than those reported in the literature. For example, Yang *et al.*[26] conducted a pharmacokinetic study by oral and i.v. administration of 0.5 and 0.01 g kg^{-1} BW curcumin to rats, respectively, and the oral bioavailability was determined to be 1%. Similarly, Li *et al.*[27] prepared curcumin liposome and performed a pharmacokinetic experiment by oral and i.v. administration of 0.1 and 0.005 g kg^{-1} BW to rats, respectively, and the oral bioavailability was shown to be 2.7%. Apparently, the high absolute bioavailability of BDMC, DMC, curcumin and total curcuminoids in the curcuminoid nanoemulsion shown in the present study can be attributed to a small particle size (12.1 nm), high dose (0.1 g kg^{-1} BW) and high encapsulation efficiency (98.8%).

Possible *in vivo* application and industrial translation

Over the years, many traditional natural products have been researched extensively in drug discovery and demonstrated to be protective against various human diseases.[28–32] Given their anti-oxidant and anti-inflammatory properties, curcuminoids from *C. longa* play a significant therapeutic role in various chronic diseases, including cancer, cardiovascular disease, Alzheimer's disease, inflammatory disorders, neurological disorders, etc.[33] However, their poor aqueous solubility, low bioavailability, instability at physiological pH and rapid metabolism limits their clinical application.[34–36] One of the best approaches for remedying this problem is to improve the bioavailability by encapsulating curcuminoids in a nanoformulation for attaining a safe toxicological profile, efficient targeting, dose reduction and effective therapeutic action.[37] Consequently, over a decade, attempts have been made to improve the pharmacokinetics, systemic bioavailability and biological activity of curcuminoid by loading curcuminoids into different types of nanoformulations.[33,37] Most importantly, many *in vivo* studies have demonstrated enhanced therapeutic efficiency because of improved bioavailability resulting from nanoformulations, as is evident from a list of review articles on both *in vitro* and *in vivo* application of curcuminoid nanoformulations.[33,38–44] In the present study, we have demonstrated a relatively high absolute bioavailability for curcuminoid nanoemulsion compared to that reported in the literature. Nevertheless, further studies are warranted to examine the anti-tumor effect of our curcuminoid nanoemulsion in rats.

Another challenge is the translation of curcuminoid nanoformulation for the manufacture of pharmaceutical grade nanocurcuminoid by adopting a scale-up process in industries. The top-down approach is a traditional scale-up process that employs NanoCrystals® technology for developing curcuminoid nanoformulations (Elan Pharma International Ltd., Shannon, Ireland).[33,37] However, this technology fails to provide good monodispersity of nanoparticles. Conversely, a bottom-up approach was successfully developed in recent years for scaling up of the poly(lactide-co-glycolide)-based curcumin nanoparticles obtained by a solid oil/water emulsion technique.[45] Moreover, several companies world-wide have manufactured and commercialized some curcuminoid nanoformulations including Nanoliposomal curcumin by NanoLiposomal Nutritionals (San Diego, CA, USA), NanoBioSphere™ complex by Life Enchancement Products, Inc. (Minden, NV, USA), Nanocurcumin (N-curcusorb) by Konark Herbals & Health Care (Mumbai, India), Nanocurcumin by SignPath Pharmaceuticals, Inc. (Quakertown, PA, USA) and Nano curcumin (Nano curcuma) by Nano Tech Miso-N (Seol, South Korea).[33,37] Thus, it is feasible that the curcuminoid nanoemulsion prepared in the present study can be commercialized by adopting an appropriate industrial scale-up process. Overall, although many studies have shown that curcuminoid nanoformulations could tackle various signaling pathways involved in the treatment of human diseases,[33,37] there is still a lack of data in clarifying the risks associated with the application of curcuminoid nanoformulation in clinical trials and for human consumption. By overcoming these issues, this fascinating polyphenol can emerge as a potential botanic drug for treatment of various human diseases.

CONCLUSIONS

In conclusion, an HPLC method was developed to separate BDMC, DMC and curcumin in *C. longa* within 10 min with flow rate of

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1 mL min^{-1}, column temperature of 35 °C and detection wavelength of 425 nm, employing an Eclipse XDB-C18 column (inner diameter 150 × 4.6 mm) with a gradient mobile phase of 0.1% formic acid in water (A) and acetonitrile (B). A high stability of curcuminoid nanoemulsion was prepared. At the same dose of 0.1 g kg^{-1} BW, the oral bioavailability of BDMC, DMC, curcumin and total curcuminoids in nanoemulsion was 34.39%, 39.93%, 47.82% and 46%, respectively, and was 4.65%, 5.49%, 9.38% and 8.7% in dispersion.

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