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Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions

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Abstract

High-speed and high-pressure homogenized O/W emulsions using medium chain triacylglycerols (MCT) as oil and Tween 20 as emulsifier, with mean droplet sizes ranging from 618.6 nm to 79.5 nm, have been successfully prepared. The enhanced anti-inflammation activity of curcumin encapsulated in O/W emulsions is evidenced by the mouse ear inflammation model. There is a 43% or 85% inhibition effect of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced edema of mouse ear for 618.6 nm and 79.5 nm 1% curcumin O/W emulsions, respectively, but a negligible effect is found for 1% curcumin in 10% Tween 20 water solution.

Keywords: Curcumin; O/W emulsions; High-speed homogenization; High-pressure homogenization; Mouse ear inflammation model

1. Introduction

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is a natural polyphenolic phytochemical extracted from the powdered rhizomes of turmeric (*Curcuma longa*) (Khanna, 1999). It has attracted considerable attention in recent years due to its great variety of beneficial biological and pharmacological activities (Duvoix et al., 2005). Besides its effective antioxidant (Sharma, 1976), antitumor (Khar, Ali, Pardhasaradhi, Begum, & Anjum, 1999), anti-inflammatory (Chan, Huang, Fenton, & Fong, 1998), anticarcinogenic (Huang et al., 1994) and free radical scavenger properties (Sreejayan & Rao, 1996), it is believed that curcumin is a potent agent against many diseases such as anorexia, coughs, diabetes, hepatic disorders, rheumatism, and Alzheimer disease (Aggarwal, Kumar, & Bharti, 2003; Jain, Rains, & Jones, 2006; Ono, Hasegawa, Naiki, & Yamada, 2004). Hydroxyl groups of the benzene rings, double bonds in the alkene part, and the central β-diketone moiety are suggested to be likely responsible for the high beneficial activities of this polyphenolic molecule (Osawa & Namiki, 1985; Ruby, Kuttan, Babu, Rajasekharan, & Kuttan, 1995).

Nevertheless, the majority of the orally administered curcumin is excreted in the feces and the urine, very few is detected in blood plasma (Pan, Huang, & Lin, 1999). One reason for the low bioavailability of curcumin is that curcumin is almost insoluble in water at acidic or neutral pH, and thus is difficult to absorb (Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007). At the same time, curcumin, after oral dosing, is rapidly metabolized in the intestine to several reduced products (di-, tetra-, hexahydrocurcumin, and hexahydrocurcuminol) and their glucuronide or sulfate conjugates (Ireson et al., 2001; Lin, Pan, & Lin-Shiau, 2000; Pan et al., 1999). Attempts to improve water solubility, stability, and bioavailability of curcumin by complex formation or interaction with macromolecules including gelatine and polysaccharides (Tønnesen, Másson, & Loftsson, 2002) and phospholipid (Liu, Lou, Zhao, & Fan, 2006; Maiti et al., 2007) have been reported. The disadvantages of previous work include the

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use of alkaline conditions and organic solvents. The development of other simple and effective approaches is therefore necessary in order to improve the solubility and bioavailability of curcumin.

Emulsion-based delivery systems have been widely used in food industry to protect active ingredients against extreme conditions, to enhance their stability, to maintain their effectiveness, and to mask bad odors and bitter tastes (Madene, Jacquot, Scher, & Desobry, 2006). Nanoemulsions possess high kinetic stability due to their extremely small emulsion droplet sizes in the range of 50–200 nm, much smaller than the range from 1 to 100 μm for conventional emulsions (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005; Sonneville-Aubrun, Simonnet, & L'Alloret, 2004). High-energy emulsification methods (Walstra, 1983), such as high-shear blending, high-pressure homogenization, and ultrasonic homogenization, as well as low-energy emulsification methods like the phase inversion temperature method (Rang & Miller, 1999), can be used to prepare nanoemulsions. Various oil-soluble or water-soluble phytochemicals have already been successfully encapsulated using nanoemulsions (Garti, Aserin, Spernath, & Amar, 2003; Shefer & Shefer, 2003; Solans et al., 2005). In this paper, we have prepared oil-in-water (O/W) nanoemulsions of different sizes to encapsulate curcumin in order to improve its anti-inflammation activity, which was evaluated by using a mouse ear inflammation model.

2. Materials and methods

2.1. Materials

Curcumin (85% pure, with 11% of demethoxycurcumin and 4% of bisdemethoxycurcumin as impurities) was a gift from Sabinsa Corporation (Piscataway, NJ) and used without further purification. The chemical structure of curcumin is depicted in Fig. 1. Medium chain triacylglycerols (oil, MCT) was kindly provided by Stepan Company (Northfield, IL). Tween 20 (emulsifier) was purchased from Sigma–Aldrich Company (St. Louis, MO). Milli-Q water was used in all experiments.

2.2. Preparation and characterization of O/W emulsions

The mixtures of MCT, Tween 20, and water were mixed under magnetic stirring for 30 min at a ratio of 10/10/80 to fabricate O/W emulsions. Some emulsion premixes were subjected to high-speed homogenization (High-speed homogenizer, ULTRA–TURRAX T-25 basic, IKA Works Inc., Willmington, USA) for 10 min. Some high-speed homogenized emulsions were further homogenized by using a high-pressure homogenizer (High pressure homogenizer, EmulsiFlex-C3, AVESTIN Inc., Ottawa, Canada) for 6 cycles. The sizes of emulsion droplets can be varied by the speed and the pressure in the homogenization processes. The final pH values of these emulsions were between 5.0 and 5.5. The stability of emulsions was tested by observing the separation height of the creaming layer after 24 h. The morphology and droplet size of emulsions were measured using an inverted optical microscopy (Nikon TE 2000, Nikon Corporation, Japan) and photon correlation spectroscopy (PCS) – based BIC 90 plus particle size analyzer equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corporation, New York, NY, USA), respectively. The light source of the particle size analyzer is a solid state laser operating at 658 nm with 30 mW power, and the signals were detected by a high sensitivity avalanche photodiode detector. All measurements were made at a fixed scattering angle of 90° and temperature of 25.0 ± 0.1 °C. The normalized field-field autocorrelation function $g(q,t)$ is obtained from the intensity-intensity autocorrelation function, $G(q,t)$, via the Sigert relation (Stepanek, 1993):

$$\alpha g(q,t) = [G(q,t)/A - 1]^{1/2} \quad (1)$$

where A is the experimentally determined baseline, α is the contrast factor which is less than 1, due to the fact that only a fraction of dynamic scattering intensity falls within the correlator window and also the fact that a finite size pinhole is used in the experiment. For all particle size measurements, the measured baseline A is in agreement with the theoretically calculated baseline to 0.01%.

Two procedures were used to analyze the $g(q,t)$ versus t data. The first data analysis method utilized in this paper was William–Watts (WW) single stretched exponential function given by

$$g(q,t) = \exp[-(t/\tau)^{\beta}] \quad (2)$$

Here β is a parameter that describes the polydispersity of diffusing particles. For monodisperse emulsion droplets, $\beta = 1$ is expected; for a polydisperse system, $0 < \beta < 1$.

The diffusion coefficient D was calculated according to $D = \tau^{-1}q^{-2}$, where q is the amplitude of scattering vector defined as $q = (4\pi n/\lambda)\sin(\theta/2)$, n is the solution refractive index, λ is the laser wavelength and θ is the scattering angle. The diffusion coefficient D can be converted into mean emulsion droplet diameter d using the Stokes–Einstein equation:

$$d = \frac{kT}{3\pi\eta D} \quad (3)$$

where k is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity.

The second method used was Cumulant analysis, where $g(q,t)$ was decomposed into a distribution of decay rate Γ ($=1/\tau$) given by

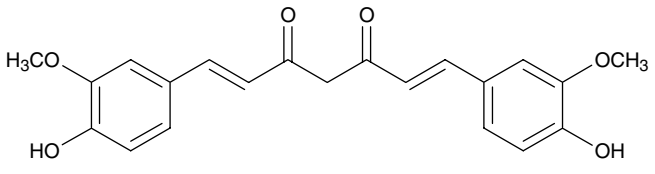

Fig. 1. Chemical structure of curcumin.

$$g(q,t) = \int G(\Gamma)e^{-\Gamma t}d\Gamma \quad (4)$$

The first two moments of the distribution $G(\Gamma)$ are as follows:

$$\Gamma = Dq^2 \quad (5)$$

$$\mu_2 = (D^2 - D^{*^2})q^4 \quad (6)$$

where D^* is the average diffusion coefficient. The polydispersity term defined in the Cumulant analysis is:

$$\text{Polydispersity} = \mu_2/\Gamma^2 \quad (7)$$

Here polydispersity has no unit. It is close to zero for monodisperse or nearly monodisperse samples, and larger for broader distribution.

2.3. Encapsulation of curcumin in O/W emulsions

Curcumin was first dissolved into hot MCT under stirring. O/W emulsions entrapped with curcumin were prepared using the method described above.

2.4. Stability of curcumin encapsulated in O/W emulsions

Ultraviolet–visible (UV–Vis) absorption spectra of curcumin encapsulated in O/W emulsions were recorded on a CARY Eclipse UV spectrometer with 1 cm optical path. The control experiments were also carried out for blank O/W emulsions and curcumin in 10% Tween 20 water solution.

2.5. Mouse ear inflammation model

Topical application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) can rapidly induce edema of mouse ear in a dose- and time-dependent manner (Huang, Smart, Wong, & Conney, 1988). Female CD-1 mice (6–7 weeks old) were orally administered with 1 mL vehicle (emulsion control containing no curcumin) or 1 mL 1% curcumin in 10% Tween 20 water solution or curcumin emulsions by gavages at 30 min before topical application of 10 μL acetone or TPA (1.5 nmol/L) in acetone. The mice were sacrificed by cervical dislocation. Mouse ear punches (6 mm in diameter) were finally taken and weighed.

3. Results and discussion

In the case of O/W emulsion preparations, stir-only and high-speed homogenization at low speed (<13,000 rpm) could not form stable emulsions, wherein the mixture of MCT, Tween 20, and water at a ratio of 10/10/80 eventually phase separated within 24 h. When high-speed homogenization was carried out at 24,000 rpm, stable O/W emulsions were obtained. After high-speed homogenization at 24,000 rpm (HS24k), emulsions were further homogenized with high-pressure homogenizer at the different pressures of 500 (HP500), 1000 (HP1000), and 1500 (HP1500) bars to achieve emulsions of smaller droplet sizes. Figs. 2a–e show the optical microscope images of various O/W emulsions measured by inverted optical microscopy. It is noted that the premixed emulsion has bigger droplet sizes and size distribution, with diameters ranging from 2 to 20 μm. In addition, this emulsion is not very stable and can phase separate within 2 h. Significantly smaller emulsion droplets can be observed in high-speed homogenized emulsion prepared at 24,000 rpm. Due to the detection limit of the optical microscope, the images of high-pressure homogenized emulsions show almost no emulsion droplets, especially at pressures as high as 1000 bar and 1500 bar.

To obtain the emulsion droplet size and size distribution, we also carried out photon correlation spectroscopy (PCS) measurements. Figs. 2f, g show the PCS results of high-pressure homogenized O/W emulsion prepared at 1500 bar and analyzed by Cumulant analysis and single stretched exponential fit, respectively. The mean emulsion diameters and polydispersities of different emulsions analyzed by these two data analysis methods were listed in Table 1. Both data analysis methods gave comparable droplet sizes, and their size discrepancy and polydispersity decreased as the homogenization pressure increased. For example, the mean emulsion droplet size difference between two fitting methods was 11% for HS24k, and decreased to only 3% for HP1500. Similarly, the polydispersities obtained from both methods also decreased with the increase of pressure. Similar homogenization pressure effects on emulsion droplet sizes were also reported by other groups using different emulsifiers (Tesch & Schubert, 2002; Seekkuarachchi, Tanaka, & Kumazawa, 2006). It is known that emulsions with smaller sizes have higher kinetic stability, which is ascribed to a larger reduction in the creaming velocity, faster Brownian diffusion, and larger steric stabilization effects for emulsion droplets (Solans et al., 2005). It is also noted from Table 1 that the emulsion viscosity increases slightly as the average droplet diameter decreases. This is because the number and the interfacial area of emulsion droplets become larger as the droplet size decreases (Nunez et al., 2000). Consequently, the interpenetration between the ethylene oxide chains of emulsifiers adsorbed on adjacent droplets is stronger, leading to an increase in emulsion viscosity. Within seven days, microscope images, emulsion droplet sizes, as well as emulsion viscosity show negligible changes for these four high-speed or high-pressure homogenized emulsions. The consistent physical properties of these emulsions with time indicate the good stability of O/W emulsions prepared.

Subsequently, two O/W emulsions prepared by high-speed homogenization at 24,000 rpm and high-pressure homogenization at 1500 bar were used to encapsulate curcumin. Although curcumin is almost insoluble in water (<10 μg/ml) (Maiti et al., 2007), it is found that 1% curcumin can be successfully encapsulated in O/W emulsions, suggesting that O/W emulsions have a high capacity to carry curcumin. On the other hand, because of the π–π^*

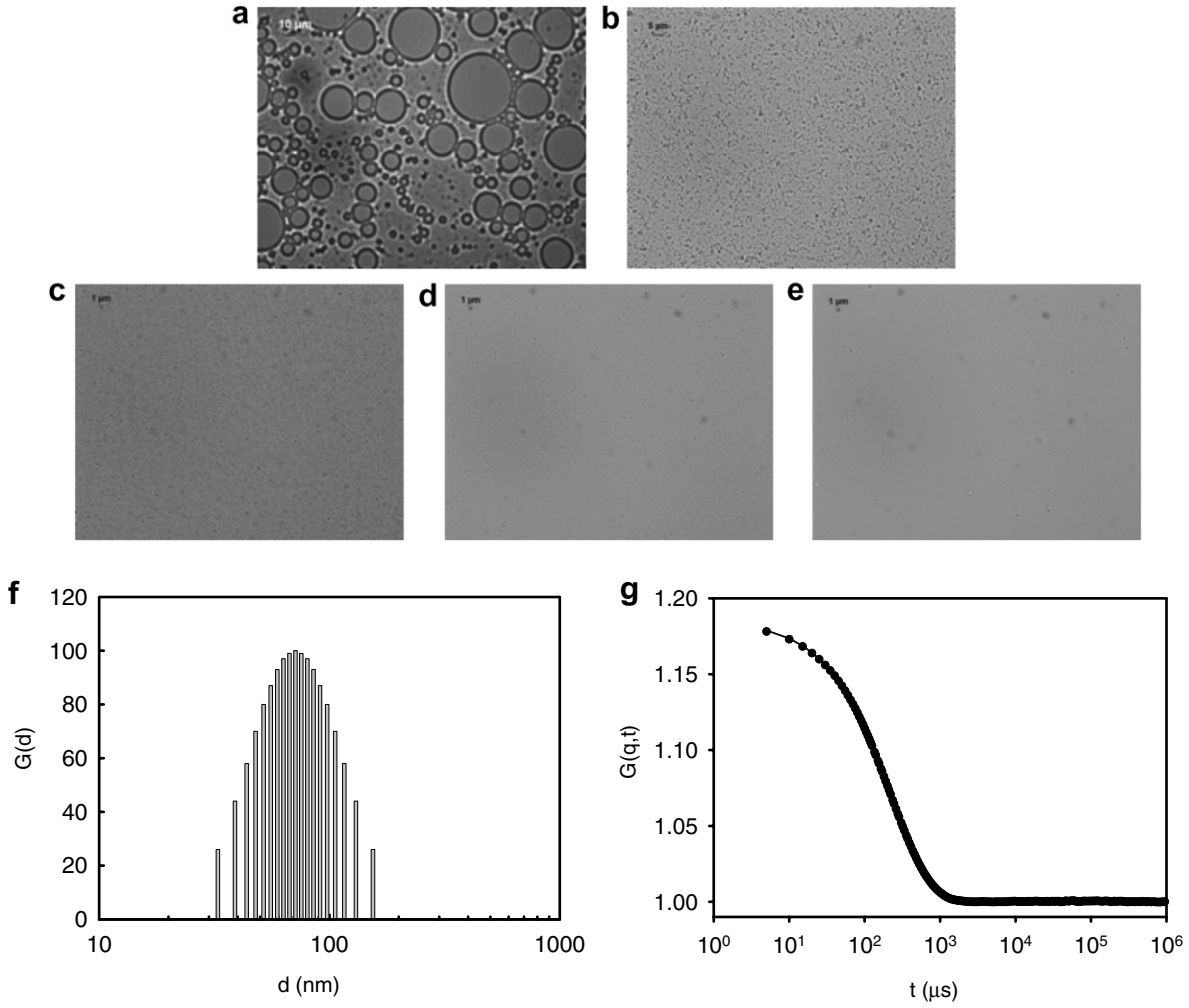

Fig. 2. Optical microscope images of O/W emulsions: (a) stir-only; (b) high-speed homogenization at 24,000 rpm; (c) high-pressure homogenization at 500 bar; (d) high-pressure homogenization at 1000 bar; and (e) high-pressure homogenization at 1500 bar; as well as photon correlation spectroscopy results of emulsion (e) analyzed by (f) Cumulant analysis; and (g) single stretched exponential fit, in which the solid circles are experimental data and the solid line is the fitting result.

Table 1
Droplet sizes and polydispersity of O/W emulsions

Emulsions	HS[a]	HP-1[b]	HP-2[c]	HP-3[d]
Mean droplet diameter by stretched exponential fits (nm)	683.2	184.7	127.0	82.1
Distribution parameter (β)	0.81	0.82	0.83	0.86
Mean droplet diameter by Cumulant analysis (nm)	618.6	174.3	120.4	79.5
Polydispersity	0.369	0.289	0.288	0.249
Viscosity (mPa s)	13.5	17.3	19.8	32.1

[a] High-speed homogenization at 24,000 rpm.
[b] High-pressure homogenization at 500 bar.
[c] High-pressure homogenization at 1000 bar.
[d] High-pressure homogenization at 1500 bar.

type excitation of the extended aromatic system, curcumin exhibits an intense absorption band in the visible region (Chignell et al., 1994; Peret-Almeida, Cherubino, Alves, Dufosse, & Gloria, 2005). We also carried out UV–Vis measurements to monitor the stability of curcumin encapsulated in O/W emulsions. Fig. 3 shows the UV–Vis spectra of O/W emulsion with curcumin homogenized by high-pressure homogenization (HP 1500) during seven-day storage. For comparison purposes, we also provided the UV–Vis spectra of emulsion control containing no curcumin and curcumin in 10% Tween 20 water solution. The nearly-unchanged absorption peak of curcumin indicates

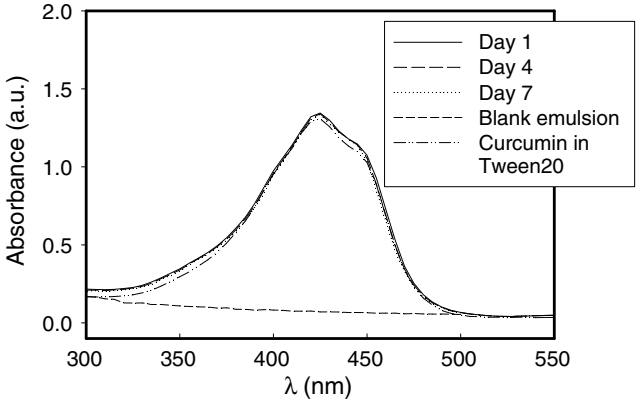

Fig. 3. UV–Vis spectra of 1% curcumin nanoemulsion prepared by high-pressure homogenization at 1500 bar after 1, 4, 7 days, as well as the blank O/W emulsion and curcumin in 10% Tween 20 water solution.

that at a pH between 5.0 and 5.5, the stability of curcumin can be maintained in O/W nanoemulsions.

Fig. 4 shows the animal test results of the anti-inflammation activity of 1% curcumin encapsulated in O/W emulsions of 618.6 nm and 79.5 nm using mouse ear inflammation model, and 1% curcumin dissolved in 10 wt% Tween 20 water solution. The oral administration of 1% curcumin in Tween 20 water solution shows little or no inhibition effect of TPA-induced edema of mouse ear. On the contrary, it is found that curcumin O/W emulsions could significantly improve the inhibition effect of TPA-induced edema of mouse ear from 43% to 85% for curcumin emulsion with droplet size of 618.6 nm and 79.5 nm, respectively. According to the previous studies (Pan et al., 1999; Lin et al., 2000; Ireson et al., 2001), it is the curcumin, not the curcumin's metabolic products

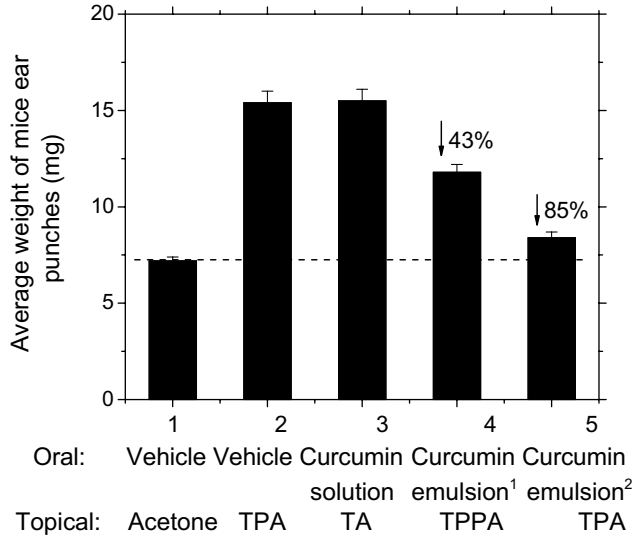

Fig. 4. The effects of oral administered curcumin nanoemulsion on TPA-induced edema of mouse ears. The vehicle is the blank O/W emulsion without curcumin. [1]High-speed homogenization at 24,000 rpm; [2]high-pressure homogenization at 1500 bar.

that have beneficial effects. Therefore, although 10 wt% Tween 20 water solution in the form of micelle (far above Tween 20's critical micelle concentration) can dissolve curcumin well and form a clear solution, Tween 20 itself cannot prevent the fast metabolism of curcumin in mouse body, thus showing no inhibition effect on the edema of mouse ear. However, oil droplets emulsified by Tween 20 cannot only disperse curcumin, but also promote the absorption of curcumin within the intestine tract due to the presence of lipids in emulsion droplets, and eventually improve the inhibition effect on the edema of mouse ear, in agreement with the beneficial effects of O/W emulsions for the oral administration of drugs (Constantinides, 1995; Uno et al., 1999). It should be pointed out here that it is the synergistic effects of both emulsion droplet size and the presence of lipid in the emulsion that provide the optimum anti-inflammation activity of curcumin. Accordingly, high-pressure homogenized curcumin emulsions containing 79.5 nm emulsion droplets exhibit higher anti-inflammation activity than high-speed homogenized curcumin emulsions containing 618.6 nm emulsion droplets.

In summary, compared with 1% curcumin in 10% Tween 20 water solution, 1% curcumin O/W emulsions show improved inhibition on the edema of mouse ear, and such anti-inflammation activity was further enhanced when the emulsion droplet sizes were reduced to below 100 nm. Further studies are still undergoing to evaluate the pharmacokinetics and biodistribution of curcumin encapsulated in O/W emulsions.

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