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Nanoemulsion of D-limonene in water system prepared by ultrasonic emulsification

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Synopsis

D-Limonene is a component of essential oil extracted from citrus fruits. This component has shown chemopreventive and therapeutic activity against a wide variety of experimental tumors, but D-limonene is unstable and lose its lemon-like flavor under normal storage condition, and it is almost insoluble in water. Therefore, studying the formation of nanoemulsion in D-limonene in water system is probably a good method to prevent the oxidation degradation of D-limonene. For the purpose of our study, we used mixed surfactant to form D-limonene-in-water emulsion, and found the best formula for forming nanoemulsion droplets with specified hydrophilic–lipophilic balance (HLB) value and droplet size. The results demonstrated that nanoemulsion droplets formed at S_o ratio of 0.4 and applied power of 18 W for 120 s under mixed surfactant at HLB values 12 and had droplet size of 20–50 nm.

INTRODUCTION

Nanoemulsions are emulsions with droplet size between 20 and 100 nm (1). Because of the small droplet size, nanoemulsions appear transparent or translucent and possess more stability against sedimentation, coalescence, flocculation, and Ostwald ripening compared with convention emulsions (2,3). The physicochemical properties of nanoemulsions are interesting for practical applications because of the small droplet size and longtime stability. Nanoemulsions are used in agrochemicals for pesticide delivery (4), in cosmetics as a vehicle for personal care or skincare products (5), in fragrance as a matrix to encapsulate the volatile compounds and controlled release which are desirable to be formulated alcohol free (6), and in beverages, to give the products an opaque appearance and suitable aroma (7).

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Using ultrasonic emulsification to prepare nanoemulsions is a recent development phenomenon (8). Ultrasonic emulsification was able to produce nanostructured droplets in emulsion with the advantage of less occurrence of "over processing" (9). Formation of nanostructured droplets are controlled by the interaction between droplet disruption and droplet coalescence, the ultrasound applied excellent shear force to droplet breakup, and the rate of droplet coalescence is determined by the mixed surfactant concentration (10). In the desired droplet size, ultrasonic emulsification can reduce the surfactant concentration and energy consumption, and the emulsions were more stable compared with homogenizer or other mechanical devices (8). There are two main mechanisms during ultrasonic emulsification (11). First, an acoustic field produces interfacial waves to break the disperse phase into the continuous phase. Second, the formation of acoustic cavitation is used to collapse microbubbles into droplets of nanometric size by pressure fluctuations.

The best nanoemulsion droplets in emulsion were prepared at optimum hydrophilic–lipophilic balance (HLB) value and optimum surfactant level (12). The proper HLB values of the surfactants are important parameters for the formation of emulsion. Nanostructured droplets in emulsion are usually formulated to enhance the stability by using a mixed surfactant because of the broad chain length distribution. Paraffin oil in water nanoemulsions have been obtained by adjusting the HLB values of the mixed surfactants Tween 80/Span 80 (2). Isohexadecane O/W nanoemulsions have been obtained in water/C12E4:C12E6/isohexadecane system at 4 and 8 wt% mixed surfactant concentration (13). Nanoemulsions containing the antioxidant astaxanthin prepared with mixed surfactant had smaller droplet size and a narrow size distribution (14).

The main objectives of this study were to gain a better understanding of the influence of mixed surfactant on the nanoemulsion droplet size by using ultrasonic emulsification and also to investigate the optimum formulation for preparing D-limonene in water nanoemulsions.

MATERIALS AND METHODS

MATERIALS

D-Limonene (RI = 1.487) was a product of Merck (Darmstadt, Germany) and used as received. Reagent grade sorbitane trioleate and polyoxyethylene (10) oleyl ether with an average HLB of 1.8 and 12.0 were supplied by Sigma-Aldrich (St. Louis, MO). Ethylene glycol used as a cosurfactant was obtained from Merck; water was deionized and Milli-Q filtered.

COARSE EMULSION PREPARATION

Emulsions consisted of D-limonene, mixed surfactant, deionized water, and cosurfactant. All emulsions were prepared in two stages. The coarse emulsion was obtained by using Polytron (PT-MR 3000, kinematica AG, Littau, Switzerland), and then further emulsified

by ultrasound process. The concentration of D-limonene was in 10 wt%, while the HLB values of mixed surfactant varied from 2 to 12. The mixed HLB values were calculated as follows: $HLB_{mix} = HLB_S \cdot S\% + HLB_P \cdot P\%$, where HLB_S, HLB_P, and HLB_{mix} were the HLB values of sorbitane trioleate, polyoxyethylene (10) oleyl ether, and mixed surfactants, and S% and P% are the mass percentages of sorbitane trioleate and polyoxyethylene (10) oleyl ether in the mixed surfactants, respectively. The HLB number of the surfactants was considered to be the algebraic average of HLB of the individual surfactant. The ratio of D-limonene to mixed surfactant was expressed in terms of S_o ratio. The cosurfactant concentration was fixed in 1%.

ULTRASONIC PROCESS

Ultrasonic process was performed by using a 20 kHz sonicator 3000 (Misonix Inc., Farmingdale, NY) with a 20-mm-diameter tip horn. The tip of the horn was symmetrically placed in the coarse emulsion, and the experiment was started at various preset ultrasonic nominal powers (6–51 W) for 30–300 s controlled by the software of the device. Each experiment was triplicated.

DROPLET SIZE DETERMINATION

Emulsion droplet size was determined by dynamic light scattering using Nanotrac 150 (Microtrac, Inc., Montgomeryville, PA). To avoid multiple scattering effects, all emulsion samples were diluted to 10% with deionized water before the measurement. Information about emulsion droplet size was obtained via a best fit between light scattering theory and measured droplet size distribution. A refractive index of 1.487 was used for D-limonene. Emulsion droplet size results are an average of three measurements and are quoted as the mean diameter (MD). The MD is calculated using the volume distribution data and is weighted to the smaller droplets in the distribution. This value is related to population or counting of droplets.

$$\mathrm{MD} = \sum \frac{(V_i d_i^2)}{(V_i d_i^3)}$$

TRANSMISSION ELECTRON MICROSCOPIC ANALYSIS

The morphology of the D-limonene nanostructured droplets in emulsion was visualized by using the transmission electron microscope (TEM). Samples (50 µl) were added to 200-mesh formwar-coated copper TEM sample holders (EM Sciences, Hatfield, PA). The samples were then negatively stained with 50 µl of 1.5% (w/v) phosphotungstic acid for 10 min at room temperature. Excess liquid was blotted with a piece of Whatman filter paper. The TEM samples were observed with JEOL JSM-1200EX II TEM (Peabody, MA) equipped with 20 µm aperture at 67 kV.

RESULTS AND DISCUSSION

THE EFFECT OF ULTRASONIC APPLIED POWER

In this study, nanoemulsion droplets in D-limonene in water system were prepared by two steps. The first step was to prepare a coarse emulsion with droplet size around 20 μm. Then, ultrasonic process was used to further decrease the droplet size. There are two main mechanisms of emulsion droplet formation. First, droplet disruption is controlled by the type and amount of shear force applied to droplets as well as the droplet resistance to deformation, which is determined by the surfactant (9,10). The other is droplet coalescence. The rate of droplet coalescence is related to the droplet stability of emulsion, which is determined by the ability of the mixed surfactant to rapidly adsorb onto the surface of newly formed droplets. This pathway is governed by surfactant surface activity and concentration. In our study, ultrasonic emulsification was used as the source of shear force and the results of applied power on droplet size of emulsion are included in Figure 1. The results showed that the droplet size reached a minimum size at applied power of 18 W. A similar trend between emulsion droplet size and applied power has been observed by others for emulsions made with flaxseed oil and Tween 40 (10). Increasing the applied power of ultrasound can provide more shear force to decrease the size of emulsion droplet.

THE EFFECT OF ULTRASONIC TIME

The nanoemulsion droplet size under different ultrasonic time are shown in Figure 2. The result showed that the droplet size were around 200 nm during ultrasonic time of 120 s, but increase in the ultrasonic time would not obviously change in emulsion droplet size. Ultrasonic time was related to the thermodynamic equilibrium of emulsion system. Ultrasonic time affects the rate of adsorption of surfactants to the droplet surface and the droplet size distribution of newly formed droplet.

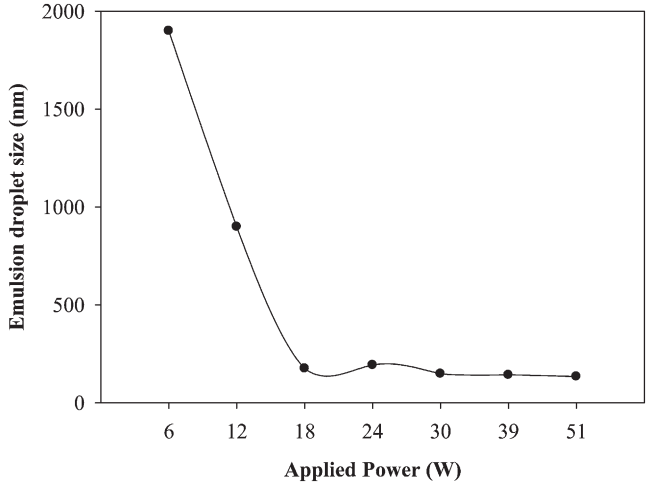

Figure 1. The effect of ultrasonic applied power on the emulsion droplet size of D-limonene in water emulsion system (S_o ratio= 0.4; ultrasonic time= 120 s).

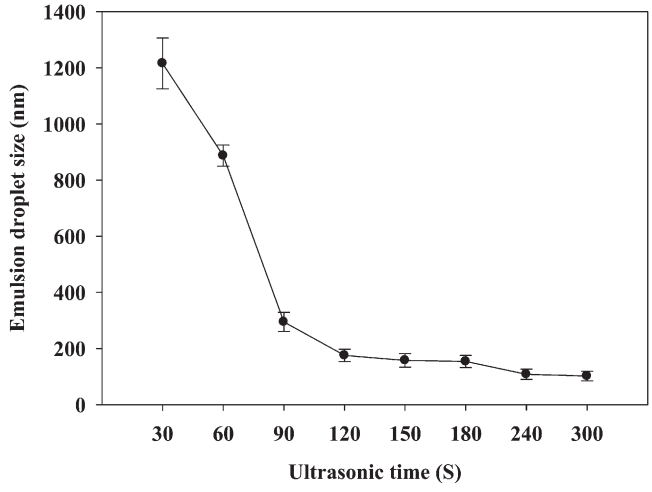

Figure 2. The effect of ultrasonic time on the emulsion droplet size of D-limonene in water emulsion system (S_o ratio= 0.4; applied power = 18 W).

THE EFFECT OF S_O RATIO

In this study, the mixed surfactant (sorbitane trioleate and polyoxyethylene (10) oleyl ether) was used as the surfactant and the effect of S_o ratio can be seen in Figure 3. At low S_o ratio, there was less mixed surfactant to absorb onto the newly formed droplet, and then increasing the S_o ratio results in the decrease of droplet size (from S_o ratio 0.2 to 0.4). However, excess mixed surfactant would not decrease the droplet size of emulsion and will interfere in the stability of emulsion. The droplet size of emulsion below 50 nm was obtained with the S_o ratio between 0.4 and 0.6, beyond that the increase in the S_o ratio

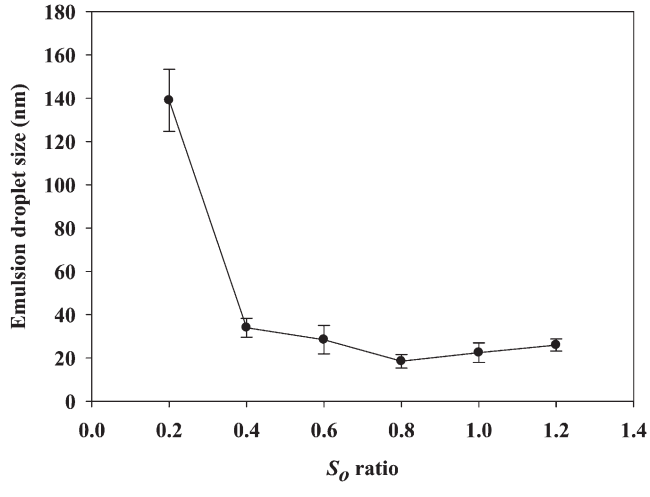

Figure 3. The effect of S_o ratio on the emulsion droplet size of D-limonene in water emulsion system (applied power = 18 W; ultrasonic time = 120 s).

did not obviously decrease the droplet size of emulsion. These results agree with other studies that discuss the relationship between surfactant concentration and droplet size of emulsion (12,15).

THE EFFECT OF HLB VALUES

The emulsion was prepared by using the mixed surfactant of sorbitane trioleate and polyoxyethylene (10) oleyl ether. The S_o ratios were adjusted to satisfy the proper HLB values for optimum emulsification condition. Emulsions with 10.0 wt% D-limonene and S_o ratio at 0.4 were prepared at different HLB values. The relationship between the droplet size of emulsions and the HLB values is shown in Table I. The results showed that droplet size of emulsions can be affected by the HLB values. The droplet size of emulsion was 332 nm at the HLB value of 2 and with an increase in the HLB value to 12, the droplet size decreased to 23 nm. The proper HLB values of mixed surfactants were key factor for the formation of emulsion droplets. During the formation of O/W emulsion, the lipophilic surfactants have more affinity to dispersed droplet of emulsion than the hydrophilic surfactant. A proper HLB value is needed to maintain the oil phase and water phase equilibrium, and with optimum HLB values, it can stabilize and narrow down the newly formed droplets during the emulsification. The appearance of emulsion is milk-like at HLB values of 2–10, and transparent or translucent to the naked eye at HLB value of 12.

TEM OBSERVATION

To observe the physical properties of the nanostructured droplet in the nanoemulsion system, TEM analysis was carried out with negatively stained samples. The results are shown in Figure 4, where phosphotungstic acid–stained D-limonene droplets are clearly

Table I
The Effect of HLB Values on the Emulsion Droplet Size and Appearance of D-Limonene in Water Emulsion System

No.	HLB values	Droplet size (nm)	Appearance
1	2	332 ± 27.3	Milk-like
2	3	335 ± 22.12	Milk-like
3	4	287 ± 14.21	Milk-like
4	5	226 ± 13.52	Milk-like
5	6	207 ± 7.33	Milk-like
6	7	175 ± 2.58	Milk-like
7	8	149 ± 3.14	Milk-like
8	9	102 ± 2.11	Milk-like
9	10	73 ± 1.29	Milk-like
10	11	54 ± 0.67	Milk-like
11	12	23 ± 0.86	Translucent

S_o ratio = 0.4; applied power = 18 W; ultrasonic time = 120 s.

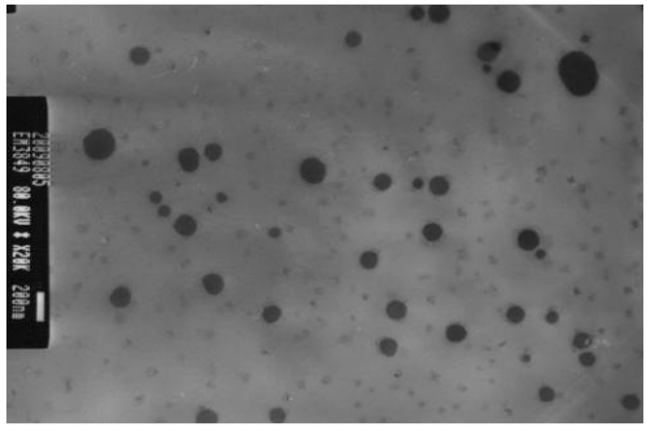

Figure 4. Visual appearance and transmission electron micrographs of nanostructured droplet in D-limonene in water emulsion system. The scale bar represents a distance of 50 nm.

visible and the droplet size correlated well with the results from droplet size analysis using Nanotrac 150 light scattering instrument. In addition, the morphology of the D-limonene droplet was spherical, and the gray parts of the droplet were D-limonene precipitation incorporated in emulsion system.

CONCLUSION

Nanoemulsion droplet can be obtained in D-limonene in water system by ultrasonic emulsification. The optimum conditions for ultrasonic emulsification of D-limonene nanoemulsions were applied power of 18 W, ultrasonic time of 120 s, and S_o ratio of 0.6 with an HLB value below 12. Nanoemulsion droplet in D-limonene nanoemulsion system was probably a solution for solving the low bioavailability and low solubility problems of D-limonene and thus might be applied as nanoencapsulated flavor and fragrance systems for industry.

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