Formation of nanostructured droplet in O/W emulsion system

(利用 O/W 模式系統來生產奈米級乳化粒子)

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Abstract

$_D$-limonene is a monocyclic monoterpene obtained from citrus fruits. This component has shown chemo-preventive and therapeutic activity against a wide variety of experimental tumors, but $_D$-limonene is unstable under normal storage condition, and it is almost insoluble in water. Therefore, studying the formation of nanostructured droplet in d-limonene in water emulsion 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 find the best formula for forming nanostructured droplet with specified HLB value and droplet size. The results revealed that nanostructured droplet formed at S_o ratio 0.4, applied power 18 W for 120 s under mixed surfactant at HLB values 12 had droplet size in 20-50 nm.

Keywords: Nanostructured droplet; $_D$-limonene; mixed surfactant.

1. INTRODUCTION

Nanostructured droplets in emulsion system are nanoemulsions with droplet size between 20-100 nm. [1]. Due to its small droplet size, it 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 its 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 it may give the products an opaque appearance and suitable aroma [7].

Using ultrasonic emulsification to prepare nanoemulsions is a recent development phenomenon [8]. Ultrasonic emulsification was able to produce nanostructured droplet in emulsion with the advantages of less occurrence of "over processing" [9]. Formation of nanostructured droplet is controlled by the interaction between droplet breakup and droplet coalescence, the ultrasound applied excellent shear force to droplet breakup, and the rate of droplet coalescence is determined by the surfactant surface activity and concentration [10]. In the desired droplet size, ultrasonic emulsification can reduce the surfactant concentration and energy consumption and the emulsions were more stable compare with homogenizer or other mechanical devices [11]. There are two mechanisms during ultrasonic emulsification [12]. Firstly, an acoustic filed produces interfacial waves to break the disperse phase into the continuous phase. Secondly, the formation of acoustic cavitation was used to collapse micro-bubbles into droplets of nanometric size by the pressure fluctuations.

The best nanostructured droplets in emulsion were prepared at optimum hydrophilic-lipophilic balance (HLB) value and optimum surfactant level [13]. The proper HLB values of the surfactants were 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% surfactant concentrations [14].

The main objectives of this study were to gain a better understanding of the relation between mixed surfactant and nanostructured droplet size by using ultrasonic emulsification, and also to investigate the optimum formulate for preparing $_D$-limonene in water nanoemulsions.

2. MATERIALS AND METHODS

2.1 Materials

$_D$-limonene (RI=1.487) was a product of Merck 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. 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, 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. Ethylene glycol was obtained from Merck, water was deionized and Milli-Q filtered.

2.2 Coarse emulsion preparation

Emulsions consisted of $_D$-limonene, mixed surfactant, deionized water and co-surfactant. All emulsions were prepared in two stages. The coarse emulsion were 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 mixed surfactant concentration was varied from 2.0 to 12.0%. The co-surfactant concentration was fixed in 1%. The ratio of $_D$-limonene to mixed surfactant was expressed in terms of S_o ratio.

2.3 Ultrasonic process

Ultrasonic process was performed by using a 20 kHz sonicator 3000 (Misonix incorporated, Farmingdale, New York) 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 to 300 second controlled by the software of the device. During emulsification, the difference of temperature from initial coarse emulsions to final emulsion was not more than 20^{o}C. Each experiment was triplicated.

2.4 Droplet size determination

Emulsion droplet size was determined by dynamic light scattering using Nanotrac 150 (Microtrac, Inc Montgomeryville, PA). In odor 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 (MN). The mean diameter 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.

$$MN = \sum \left(V_i d_i^{\,2}\right) \Big/ \left(V_i d_i^{\,3}\right)$$

2.5 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, USA). 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 transmission electron microscope (Peabody, MA, USA) equipped with 20 μm aperture at 67kV.

3. RESULTS AND DISCUSSIONS

3.1. The effect of ultrasonic applied power

In this study, nanostructured droplets in D-limonene in water emulsion 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 droplets 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 is determined by the ability of the surfactant to rapidly adsorb to the surface of newly formed droplets, this pathway is governed by surfactant surface activity and concentration. In our study the ultrasonic was used as the source of shear force and the results of applied power on the droplet size of emulsion were revealed in Fig. 1. The results showed that the droplet size reached a minimum size at applied power 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.

3.2. The effect of ultrasonic time

The nanostructured droplet size of emulsion under different ultrasonic time was showed in Fig. 2. The result showed that the droplet size were around 200 nm during 120 s ultrasonic time, but increase the ultrasonic time would not have 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.

3.3. The effect of mixed surfactant

In this study the mixed surfactant (sorbitane trioleate and polyoxyethylene oleyl ether) was used as the surfactant and the effect of S_o ratio can be seen in Fig. 3. At low S_o ratio, there were 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 be interference the stability of emulsion. The droplet size of emulsion below 50 nm was obtained with the S_o ratio between 0.4-0.6, after then increase the S_o ratio would not obviously decrease the droplet size of emulsion. This result agreed with other study they discuss the relationship between surfactant concentration and droplet size of emulsion [15].

3.4. The effect of HLB values

The emulsion was prepared by using the mixed surfactant of sorbitane trioleate and polyoxyethylene 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 was shown in Table1. The results showed that droplets size of emulsions can be effect by the HLB values. Droplets size of emulsions was in the 332 nm at the 2 of HLB values after then increase the HLB values to 12 the droplet size of emulsion decrease 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 values is needed to maintain the oil phase and water phase equilibrium, and in the optimum HLB values it can stabilize and narrow down the newly formed droplets during the emulsification. The photograph of nanoemulsion at different HLB values using the ultrasonic emulsification was shown in Fig. 4. The appearances of emulsions appear milk-like color at HLB values 2 to 10, and transparent or translucent to the naked eye at HLB values 12.

3.5 TEM observation

In order to observe the physical properties of the nanostructured droplet in the nanoemulsion system, TEM analysis was carried out with negatively stained samples. As the result was showed in Fig.5, phosphotungstic acid-stained $_D$-limonene droplets were clearly 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.

4. Conclusion

Nanostructured 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 at 18 W, ultrasonic time of 120 s and S_o ratio of 0.6 under HLB value at 12. Nanostructured droplet in $_D$-limonene nanoemulsions system was probably a solution for solving the oxidation and low bioavailability problems of $_D$-limonene and thus might be applied as nano-encapsulated flavor systems for industry.

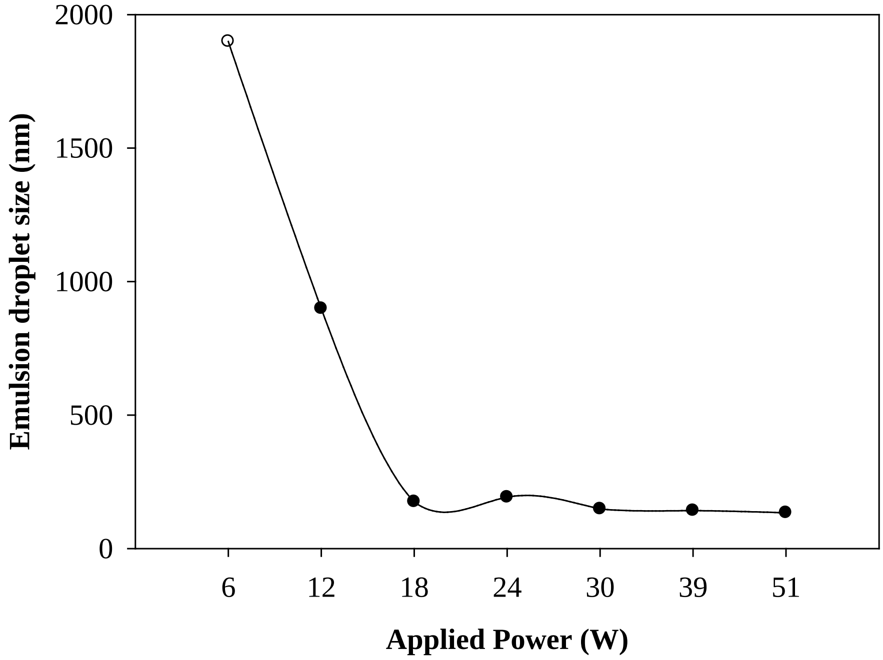

Fig. 1. Effect of applied power on the emulsion droplet size of $_D$-limonene in water emulsion system. Emulsion composition was an aqueous solution of mixing surfactant emulsified with 10wt% d-limonene. (S_o ratio= 0.4; Ultrasonic time= 120 s)

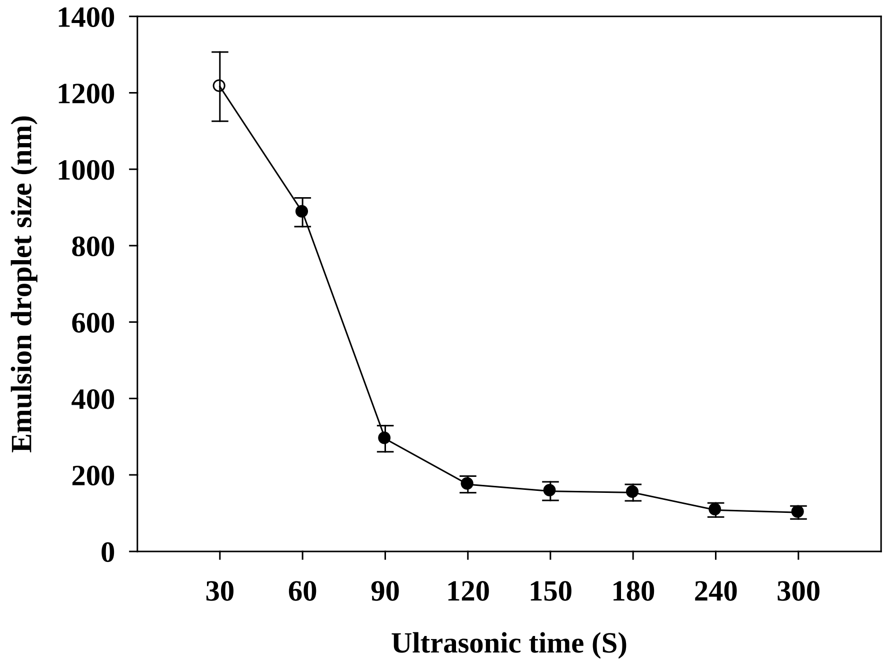

Fig. 2. Effect of ultrasonic time on the emulsion droplet size of $_D$-limonene in water emulsion system. Emulsion composition was an aqueous solution of mixed surfactant emulsified with 10 wt% d-limonene. (S_o ratio= 0.4; Applied power= 18W)

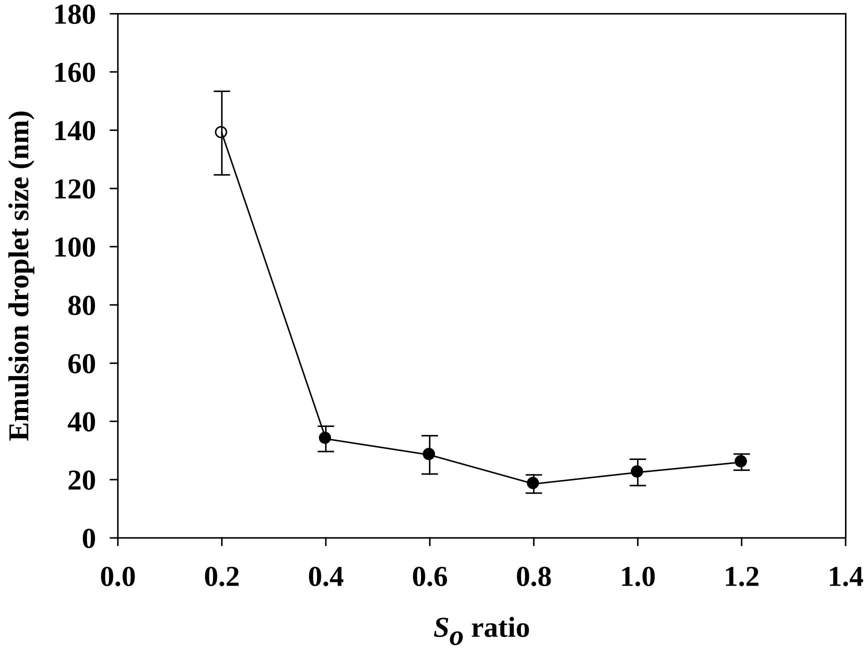

Fig. 3. Effect of S_o *ratio* on the emulsion droplet size of $_D$-limonene in water emulsion system. Emulsion composition was an aqueous solution of mixing surfactant emulsified with 5.0 wt%, 10 wt% and 15wt% d-limonene. (Applied power= 18W; Ultrasonic time= 120 s)

Table 1. Effect of HLB values on the emulsion droplet size of $_D$-limonene in water emulsion system. Emulsion composition was an aqueous solution of mixing surfactant emulsified with 10 wt% d-limonene. (S_o ratio=0.4; Applied power=18W; Ultrasonic time= 120 s)

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

HLB=2 3 4 5 6 7 8 9 10 11 12

Fig. 4. Photograph of the nanoemulsions prepared by the ultrasonic emulsification under different HLB values. Emulsion composition was an aqueous solution of mixed surfactant emulsified with 10 wt% d-limonene. (S_o= 0.4; Applied power= 18W; Ultrasonic time= 120 s).

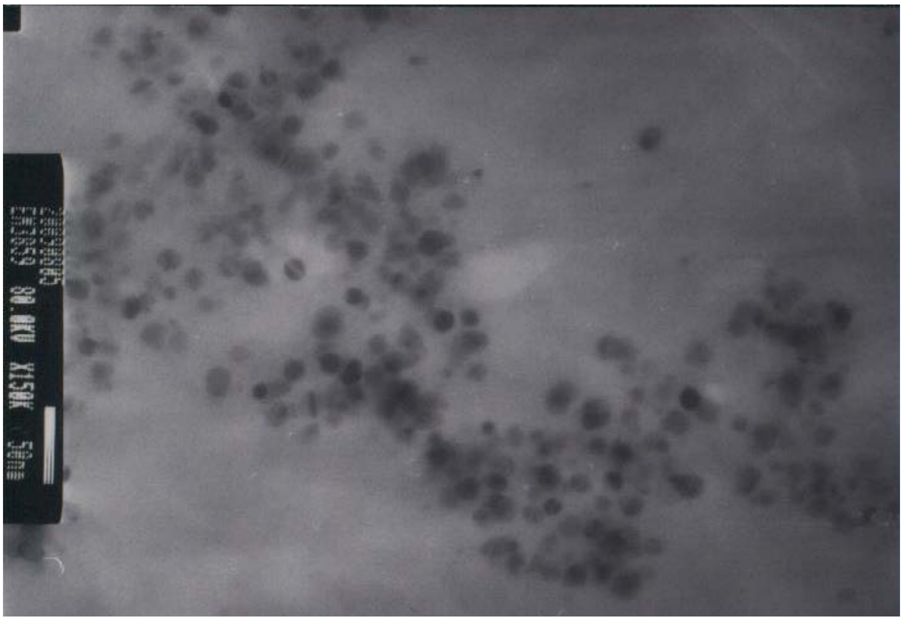

Fig. 5. Visual appearance and transmission electron micrographs (TEM) of nanostructured droplet in $_{D}$-limonene in water emulsion system.

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