Application of direct sonication in water-CO_2 system

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Abstract

The object of this study is to introduce a new technique applying direct sonication in water-carbon dioxide (CO_2) system for the extraction of water soluble natural yellow pigments, rapid production of liposomes or nanobubbles. The effects of different parameters including entrainers (water, or aqueous ethanol), temperature (5-25 °C), pressure (8-14 MPa), and sonication time (0-200 s) on relevant the final product (extraction yield, liposome or nanobubbles) were examined. The efficacy of direct sonication was evaluated by analyzing particle size distribution (PSD), scanning electron microscope (SEM) and transmission electron microscopy (TEM). Direct sonication to the water/CO_2 two-phase system caused rapid physical mixing between the water and CO_2 phases including micro-phase separation and cavitation which speed-up the mass transfer may be responsible for the high yielding the natural pigment extraction, or rapid production of liposomes or nanobubbles.

Keywords

Direct sonication, water-carbon dioxide system, extraction, liposome, nanobubble

1. Introduction

The food, cosmetics, and pharmaceutical industries are the largest users of the organic solvents, since, to extract functional components from natural products, or to produce functional micro/nanocapsules, several processing steps are performed [1-4]. Therefore, it is highly recommended to reduce these toxic organic solvents below the maximum residue limit to increase the safety level. Moreover, high temperatures are required for solvent evaporation, which, actually, should be avoid to reduce the degradation of the active molecules [1-4]. As a solution to this problem, high-pressure CO_2 (CO_2) could be an excellent alternative, typically replaces nonpolar solvents such as hexane or toluene in terms of solubility. CO_2 is particularly appealing since is low-cost, inert, non-toxic and most importantly, the density and solvent

power of CO_2 can be modulated simply by changing the pressure and temperature and in this way, controlled. It has been successfully adopted for the production of micro- and nanoparticles, or extraction of functional components from natural products. The affinity of nonpolar CO_2 can also be diverted to polar components such as crocins by adding a small amount of entrainer (co-solvent or modifier), such as water, ethanol, or aqueous ethanol. In this case, instead of adding an entrainer to the CO_2 upstream (as co-solvent), compressed liquid CO_2 is added to the entrainer (as modifier), which is well known as CO_2-expanded liquid (CXL), used for various applications, including extraction, reaction, and separation. Additionally, the application of direct sonication to the high-pressure CO_2 system has been well documented for the extraction [1,2] or production of high-yielding liposomes [4]. Direct sonication of the water/CO_2 two-phase system caused rapid physical mixing between the water and CO_2 phases, including micro-phase separation and cavitation, which may speed up the mass transfer at lower frequencies, typically 20 kHz.

Therefore, the objective of this work focus on the effects of direct sonication using the device that combines ultrasonic waves and high-pressure carbon dioxide technology conducting three experiments: 1) extraction of natural dyes, 2) production of high-concentration nanobubbles, and 3) production of liposomes. The effects of factors (pressure, temperature, ultrasonic irradiation time) on the number concentration of nanobubbles and the average particle size were investigated.

2. Experimental

2.1. Materials

Dried gardenia fruits (native to China) were purchased from S & B Foods Co., Ltd, Tokyo, Japan . The skins were peeled off and the fruit pulp was powdered (aver-age particle size, 75.4 μm) with a 6750 Freezer/Mill (SPEX CertiPrep Co. Ltd., Metuchen, NJ, USA).

Table 1
Chemicals used in this work.

Component	Source	Grade	Purity (wt%)
Ethanol	Wako Pure Chemical Industries	analytical-grade	>99.5
Methanol		HPLC-grade	>99.7
Lecithin from soybean		-	>99.7
Cyclosporin A		-	>99.7
Crocin-1 and crocin-2	ChemFaces Biochemical Co., Ltd.	-	>98%
High-purity CO_2	Fukuoka Sanso Co. Ltd	-	>99%

2.2. Apparatus and Procedure

A schematic diagram of the apparatus used for producing liposomes with liquid CO_2 and direct ultrasonication is shown in Figure 1. The high pressure cell (inner volume, 150 cm^3; dimensions, 34 mm i.d. × 165 mm height;) was equipped with a titanium ultrasound horn located at the upper part of the cell. The ultrasound horn was controlled by an ultrasonic processor (VC-750, Sonic and Materials Inc.), which was capable of producing ultrasonic waves at a frequency of 20 kHz with a maximum power of 500 W. Constant amplitude of the ultrasonic irradiation was achieved by automatically adjusting the power supply.

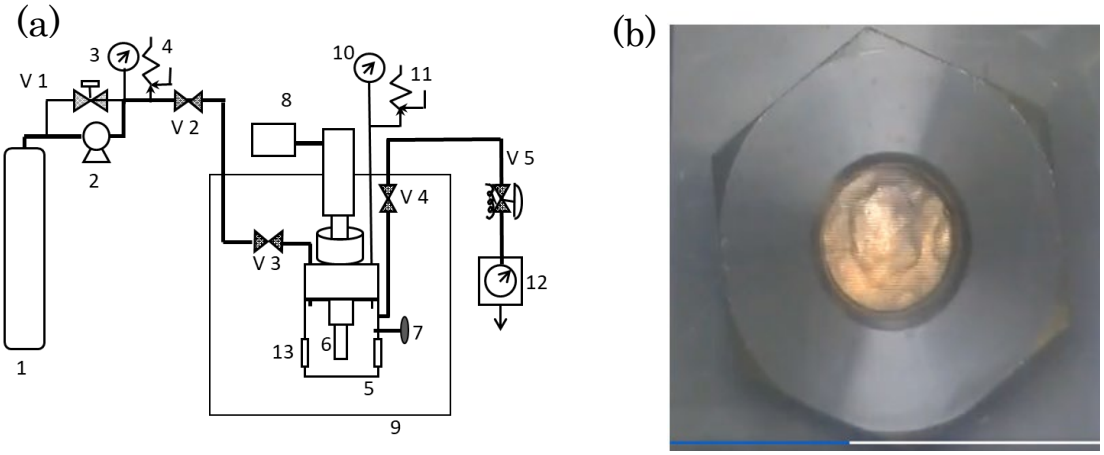

Figure 1. (a) Schematic diagram of the apparatus for liposome production by high pressure CO2 with direct ultrasonication (HPC-D). (1) gas cylinder; (2) pump; (3) pressure gauge; (4) safety valve; (5) high-pressure cell; (6) ultrasonic horn; (7) thermocouple; (8) ultrasonic processor; (9) water bath; (10) pressure gauge; (11) safety valve; (12) gas flow meter; (13) sapphire windows; (V1 and V5) back pressure regulators; (V2, V3, V4) stop valves. (b) Visual observation of high pressure cell using high pressure CO_2 methods

The experimental procedure was as follows. First, for liposome, 25 ml to 50 ml of phospholipid aqueous suspension with concentrations from 0.04 wt% to 0.4 wt% with or without drug Cyclosporin A (CsA), 0.01 g, or as for extraction, Gardenia fruit pulp milled powder (10 mg) and 10 mL of the modifier, ethanol or water, or 50 to 80% of aqueous ethanol solution, or as for nanobubbles, 25 ml of water, respectively, were loaded into the high pressure cell that was then sealed. The high pressure cell was immersed into a water bath controlled at a temperature from 25 ºC to 60 ºC. After the high pressure cell reached the target temperature, liquid CO_2 was delivered from the top of the high pressure cell with a HPLC pump coupled with a cooling unit (SCF-get, JASCO) until the target pressure was achieved. The temperature of the high pressure cell was maintained to within ± 0.1 ºC of the target temperature. After achieving the target temperature, CO_2 was introduced into the high pressure cell that was subsequently purged and sealed at 0.1 MPa. Ultrasonication was conducted at an amplitude of

25 % and performed in intervals (on time: 5 s; off time: 10 s) to prevent thermal vesicle disruption. This ultrasonication cycle was conducted for a total of 0 s, 25 s, 50 s, 75 s, 125 s, 200 s, 250 s and defined as the ultrasonication time. The amount of CO_2 released during the depressurization was measured by a gas flow meter. Then, contents were collected from the high pressure cell and filtered with a membrane filter (pore size of 0.45 μm).

The resulting liquid as for extract was analyzed by HPLC-UV for quantitative determination of crocin-1 and crocin-2. As for liposome, the filtrate was further analyzed to determine partial size and observation of particle morphology. The liposome yield of the experiments was evaluated with eq. (1):

$$\text{Liposome yield (\%)} = (1 - C_{remain}\ [g] / C_{initial}\ [g]) \times 100 \quad (1)$$

where $C_{initial}$ is the initial phospholipid mass before the CO_2 treatment and C_{remain} is the mass of phospholipid remaining in the reactor after the CO_2 treatment.

As for the polydispersity index of nanobubbles, they were represented by the Span value. A small Span value means that the size variation of nanobubbles are small. The Span value was calculated from Eq. (2) from the particle sizes d0.1, d0.5, and d0.9, which correspond to the integrated values of the particle size distribution of 10%, 50%, and 90%.

$$Span=(d_{0.9}-d_{0.1})/d_{0.5} \quad (2)$$

In order to confirm the existence of nanobubbles, the nanobubble suspension was frozen and examined whether it affected the number concentration of nanobubbles. Nanobubble water prepared by the combined ultrasonic pressure dissolution method was immediately frozen at -18 ° C for 1 day. After freezing for 1 day, it was thawed slowly at room temperature, and the number concentration of nanobubbles after thawing was evaluated by nanosite LM10 (manufactured by Malvern Panalytical).

3. Experimental results

3.1 Effect of direct sonication in water-CO_2 system on Extraction Concentration

The effect of direct sonication in water-CO_2 system using of different modifiers such as, pure ethanol (CXE) or water (CXW), or 50 or 80% of aqueous ethanol solution (CXE50% and CXE80%, respectively), on the extraction concentration of crocin-1 and crocin-2 was tested at 25 °C and 10 MPa and compared with the conventional extraction using methanol, as shown in Figure 2a,b. The results show that the concentrations of crocin-1 and crocin-2 were significantly increased with the addition of direct sonication (DS) in CXE50% + DS (12.91 ± 0.75 and 0.52 ± 0.03 μg/mL for crocin-1 and crocin-2, respectively; $p < 0.05$) or CXE 80% + DS (13.63 ± 0.52 and 0.51 ± 0.05 μg/mL for crocin-1 and crocin-2, respectively; $p < 0.05$) extraction system.

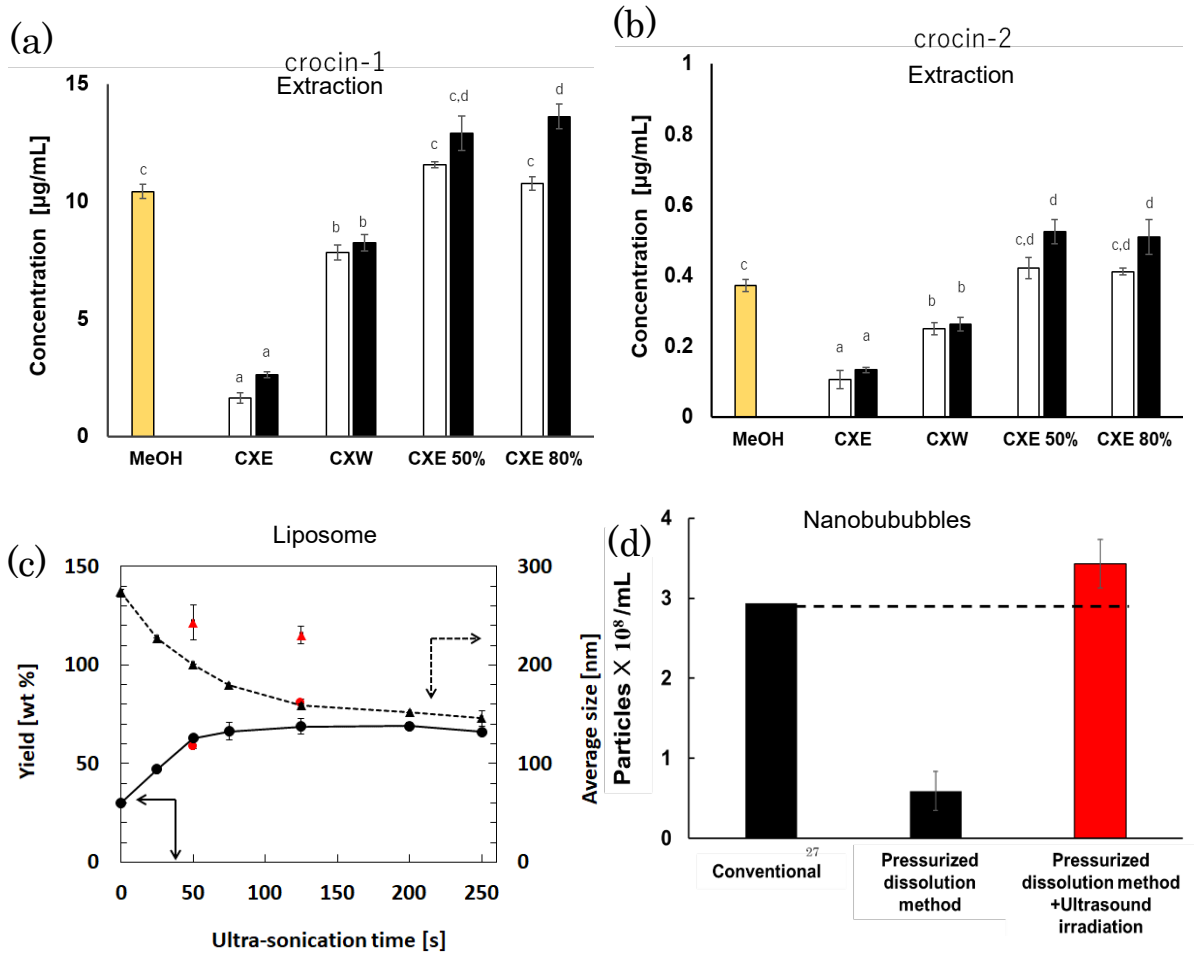

Figure 2. **(a,b)** CO_2-Expanded Liquid (CXL) Extraction with (■) or without (□) direct sonication (125 s, 20% amplitude) at 25 ° C and 10 MPa using different modifiers for crocin-1 and crocin-2. Error bars represent means ± SE (n = 3). Letters on top of the columns represent significant differences among methods at the 0.05 level. **(c)** Liposome yield (●) and average particle size (▲) as a function of ultrasonication time obtained from HPC-D and HPC-I methods conducted at 25 ºC and 6.8 MPa. Black symbols: HPC-D, orange symbols: HPC-I method. **(d)** Number concentration of nanobubbles prepared by each manufacturing method. [Preparation conditions] Pressure dissolution method: temperature 25 ° C, pressure 8 MPa, amount of water 25 mL, Pressurized dissolution method with ultrasonic waves: temperature 25 ° C, pressure 8 MPa, amount of water 25 mL, Ultrasonic time 125 s.

Figure 2c shows liposome yields and average particle sizes as a function of ultrasonication time obtained from the high pressure CO_2 with direct (HPC-D) and indirect (HPC-I) ultrasonication methods. Similarly, Figure 2d shows the number concentration of nanobubbles prepared by each manufacturing method (pressurization dissolution method and pressure dissolution method combined with ultrasonic waves). The number concentration of

nanobubbles prepared by the combined ultrasonic pressure dissolution method was about 6 times higher than that of the pressure dissolution method. The increase in yield, or the decrease in liposome particle size or high concentration of nanobubbles with direct ultrasonication indicates mechanical effects such as shock waves being formed during symmetric cavitation, or by microjets forming during asymmetric cavitation that cause physical disturbances that may increase mass transfer, or reduce the particle size of the liposomes as reported in previous studies. Irradiation of ultrasonic waves may violently disrupt the interface between carbon dioxide and water, increasing the contact area between carbon dioxide and water. As a result, the amount of carbon dioxide dissolved in water increased compared to when ultrasonic waves were not applied, and the difference in the amount of carbon dioxide dissolved during pressurization and depressurization became large, resulting in a high concentration of nanobubbles. This result indicates that by combining the pressure dissolution method with ultrasonic waves, it is possible to prepare nanobubbles having a smaller average particle size and a uniform particle size than the pressure dissolution method.

6. Conclusion

Based on the results of the three experiments conducted in this research (extraction of natural pigments, preparation of high-concentration nanobubbles, preparation of liposomes), it was revealed that the combination of high-pressure CO_2 and direct ultrasonication is an easy and effective method for extraction or efficiently producing high yields of submicron liposomes with efficient drug loading and encapsulation efficiency or nanobubbles which can be applied to various fields such as medicine and food. It is considered that this technology can contribute to the development of environment-friendly extraction technology and nanocapsule preparation technology that do not use harmful organic solvents.

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