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Preparation of micro particles of functional pigments by gas-saturated solution process using supercritical carbon dioxide and polyethylene glycol

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Abstract−Particle design is presently a major development of supercritical fluids applications, mainly in the paint, cosmetic, pharmaceutical, and specialty chemical industries. The particles from the gas-saturated solutions (PGSS) process were used to micronize the functional compounds, fucoxanthin and astaxanthin. Fucoxanthin was extracted from brown seaweed using supercritical carbon dioxide (SC-CO_2) at 20 MPa and 45 ºC. The particle formation of functional pigments with biodegradable polymer, polyethelene glycol (PEG) was performed by PGSS using SC-CO_2 in a thermostatted stirred vessel. Different temperatures (40 and 50 ºC) and pressures (10-30 MPa) were applied to optimize the conditions for the formation of functional pigment particles. Two nozzles of different diameter (250 and 300 μm) were used for PGSS and the reaction time was 1 hr. The average diameter of the particles obtained by PGSS at different conditions was about 0.78-1.42 μm.

Key words: Micro Particles, Particles from Gas-saturated Solutions, Polyethelene Glycol, Astaxanthin, Fucoxanthin

INTRODUCTION

Pharmaceutical industries always need fine particles without using organic solvents [1]. Traditionally, some methods are used for producing fine particles, including milling, grinding, spray drying, and recrystallization from solution. But most particles using these methods have some drawbacks such as the degradation of the product in mechanical treatments because of high temperature and toxic solvents. Nowadays other promising new technologies such as rapid expansion of supercritical solutions (RESS), supercritical anti solvent (SAS), particles from gas saturated solutions (PGSS) etc., are widely applied for micronization of functional materials. These techniques consume less energy and are cheaper. Moreover, the final product is free of solvents. One of the solvents, supercritical carbon dioxide (SC-CO_2), is widely used in these techniques as a green and inexpensive solvent [2-6]. Particle formation using SC-CO_2 is important for drug delivery systems and will most likely be the next major commercial application area [7]. Supercritical fluids have been successfully used to obtain composites or encapsulates, which comprise an active compound loaded into a matrix of a carrier material, in order to improve product preservation as well as controlling the dissolution rate of the active compound. The bioavailability, the ratio of drug absorbed in target area by body to initial dosage, can be improved by decreasing the particle size and maximizing the surface area, which leads to an increasing of dissolution. Small particles of pharmaceuticals with a narrow particle size distribution play a vital role in the design of conventional drug delivery systems like tablets, capsules, injections; biphasic drug delivery systems like suspension and emulsion and the controlled drug delivery systems of implants, transdermals, microemulsions, and nanoparticulates [8-14].

The PGSS process is a particle formation method based on the use of SC-CO_2. This process consists in saturating a solution containing the solute of interest with SC-CO_2. The saturation is accomplished by mixing the solute and the CO_2 under supercritical conditions by means of a static mixer or other contacting device. Afterwards, the gas-saturated solution is expanded down to atmospheric pressure through a nozzle. During the expansion, the gas dissolved into the solution is suddenly vaporized, enhancing solution atomization. Moreover, the intense cooling due to Joule-Thomson effect during CO_2 expansion promotes particle formation. Both effects make it possible to obtain particles with average sizes in the micrometer range and controlled particle size distributions [15]. In the PGSS process, micro partcles can be produced with a narrow size distrbution that are vital for food applications, free of solvent and other additives [2]. Further, with this process it is possible to produce different morphologies of particles like spherical and completely solid, spherical and hollow and distorted or agglomerated particles [16]. Many substances have been successfully micronized by PGSS, and this technology has entered industrial application [17].

Polyethylene glycol (PEG) is one of the commonly used compounds in the pharmaceutical industry because of its hydrophilic nature. PEG is available in different states such as liquid or solid depending on the molecular weight. In the solid state, PEG is mainly used in particle formation for pharmaceutical applications, such as drug carriers [1]. The functional compounds, fucoxanthin and astaxanthin, are the carotenoids found in the animal and plant kingdoms. It is well known that these compounds have potential applications in both pharmaceuticals as anti-cancer agent and in the food industry [18,19]. Brown sea weed (*Undaria pinnatifida*) contains carotenoids in which fucoxanthin is the principal pigment. Fucoxanthin is a fat soluble pigment that can be easily extracted with oil using SC-CO_2 [20]. The aim of this study is to develop and optimize a continuous process for the formation of particles of functional pigments, fucoxanthin from brown seaweed obtained by SC-CO_2 extraction and standard astaxanthin.

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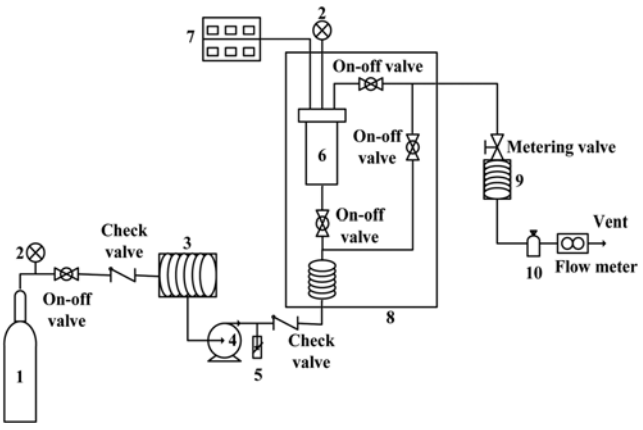

Fig. 1. Flow diagram of the supercritical fluid extraction unit.

1. CO_2 tank
2. Pressure gauge
3. Chiller
4. Pump
5. Safety valve
6. High pressure vessel
7. Temperature indicator
8. Electric oven
9. Water bath
10. Separator

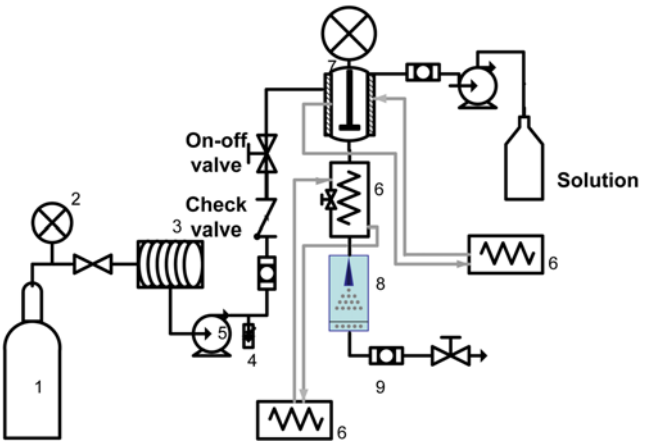

Fig. 2. Schematic diagram of PGSS process.

1. CO_2 tank
2. Pressure gauge
3. Cooling bath
4. Safety valve
5. Pump
6. Heat exchanger
7. High pressure vessel
8. Separator
9. Filter

EXPERIMENTAL

1. Materials

Brown sea weed was provided by Chungho Seafood Co. Ltd., Gi-Jang, Korea. Standard astaxanthin, fucoxanthin and Carrier PEG 8000 were purchased from Sigma-Aldrich. The carbon dioxide (99.99% pure) was supplied by KOSEM, Korea. All other chemicals used in different analysis were of analytical or HPLC grade.

2. Sample Preparation

Brown seaweeds were dried in a freeze dryer for about 48 hrs. The dried sample was mechanically crushed and sieved in 500 μm by mesh. The sieved sample was used for the extraction of oil by SC-CO_2 in order to use its functional pigment, especially fucoxanthin, for the preparation of particle.

3. SC-CO_2 Extraction

Brown seaweed oil with lipid soluble fucoxanthin was extracted by SC-CO_2. The setup of the laboratory scale supercritical fluid extraction process is shown in Fig. 1. Brown seaweed sample (12 g) was loaded into the 50 mL stainless steel extractor. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with a cap, another layer of cotton was used at the top of the sample. CO_2 was pumped at constant pressure into the extraction vessel by high pressure pump up to the desired pressure for 1 hr. The pressure of CO_2 was maintained by a digital pressure controller. An electric oven was used for maintaining the temperature of extractor. The flow rate of CO_2 was constant at 24 g/min for all extraction conditions and CO_2 volume passing through the apparatus was measured with a gas flow meter. The oil extracted by SC-CO_2 was collected by a glass separating vessel. The temperature and pressure for SC-CO_2 extraction were 45 °C and 20 MPa.

4. Particle Formation

The experiments were carried out using PEG and functional pigments (standard astaxanthin and extracted oil) with different pressures, melt temperatures, nozzle size and stirring speed. The schematic diagram of PGSS process used in this study is shown in Fig. 2. A PGSS experiment began by delivering SC-CO_2 to the precipitation chamber until the desired pressure was reached. Polyethylene glycol and pigment samples (0.3 g of sample/5.7 g of PEG) in the chamber were melted by SC-CO_2 and mixed by stirred heel. These experiments were carried out at 40 to 50 °C and pressures of 10 MPa to 30 MPa. The mixture was stirred at 200-400 rpm and the nozzle size was 250 and 300 μm. The duration for reactions was 1 hr. After reaction, functional pigment with PEG was delivered through the nozzle and collected in a separator.

5. Analysis of Particle by Scanning Electron Microscope (SEM)

Samples of the powder of functional compounds with PEG on the metallic frit were observed by a scanning electron microscope (S-2400, Hitachin, USA). The SEM samples were covered with 250 Å of gold using a sputter coater. Particles sizes were measured from SEM images using the Sigma Scan Pro image analysis software.

6. Analysis of Particle Size by Particle Size Analyzer (PSA)

The size distribution of the powder was measured by particle size analyzer (LS 13320, Beckman coulier, USA). The result from the analysis is the relative distribution of volume of particles in the range of size classes. From this basic result the statistics of the distribution are calculated. The frequency curve is useful for displaying the results to show the peaks in the graph. The peak of the frequency curve gives the modal diameter, the most commonly occurring particle diameter.

7. HPLC Analysis for Functional Pigments

Fucoxanthin and astaxanthin measurements were performed with a Waters HPLC equipped with a 600E system controller, a 484 UV/VIS detector and a Eclipse Plus C18 column (5 μm, 4.6×250 mm, Agilent, USA). Fucoxanthin was analyzed according to Wright et al. [21] and Barlow et al. [22]. Astaxanthin was analyzed by the method of Guillou et al. [23]. The mobile phase consisting of methanol : acetonitrile : ethyl acetate (80 : 10 : 10, v/v) and acetonitrile : dichloromethane : methanol : water : propionic acid (71 : 22 : 4 : 2 : 1, v/v) was used for fucoxanthin and astaxanthin, respectively. Both mobile phases were eluted 1 mL/min as an isocratic method. Fucoxanthin and astaxanthin were detected at the wavelength of 449 and

486 nm, respectively. The solvents were filtered through a 0.25 μm advantec filter prior to use and degassed by sonicator. The amounts of fucoxanthin and astaxanthin in PEG were measured based on the peak area of the standard fucoxanthin and astaxanthin, respectively.

RESULTS AND DISCUSSION

1. SC-CO_2 Extraction of Oil from Brown Seaweed

The oil obtained by SC-CO_2 extraction from brown seaweed at 20 MPa and 45 °C was 0.53 g/12 g of brown seaweed (data not shown). The increase of the extraction yield was not significant over 50 min extraction time. The effect of supercritical fluids on oil extraction from different sources at various conditions has been reported in many research works [20,24-26].

2. Characterization of Reformed Particle Obtained by PGSS

2-1. Analysis of SEM Images

This process was designed for making particles of materials that absorb supercritical fluids at high concentrations. Several experiments were performed at different temperatures in supercritical states using two different nozzles, 250 and 300 μm, for PEG 8000. The particles produced by PGSS were characterized by SEM. Fig. 3 shows SEM images of functional particles with PEG obtained by SC-CO_2 treatment at different pressures and temperatures. These results can be explained in detail with the help of the various parameters such as the temperature, pressure, nozzle size etc. Almost all particles obtained by PGSS using SC-CO_2 were spherical which leads to free-flowing powder and prevention of clogging of the nozzles during spraying of functional materials. Cocero et al. [27] also reported similar results obtained by PGSS for biopesticide encapsulation.

2-2. Particle Size Analysis by PSA

Fig. 4 shows the size distribution of the functional particles with PEG 8000 obtained by PGSS using SC-CO_2 under different conditions. On the x axis the particle size can be read, the corresponding value on the y axis represents the percentage of the sample with this particular diameter. In this study, the particle size was found to be 3 μm. The distribution of the particles shows a bimodal size, which can be explained by the effect of the strong expansion and the low temperature at the beginning of the process producing a small particle size. After a short time, the expansion strength was reduced and the temperature increased, allowing the production of another size of particle. The average particle size of PEG before PGSS process was almost 400 times bigger than that of PEG obtained by PGSS process using SC-CO_2. The average sizes of the functional particles with PEG obtained by PGSS at different conditions are shown

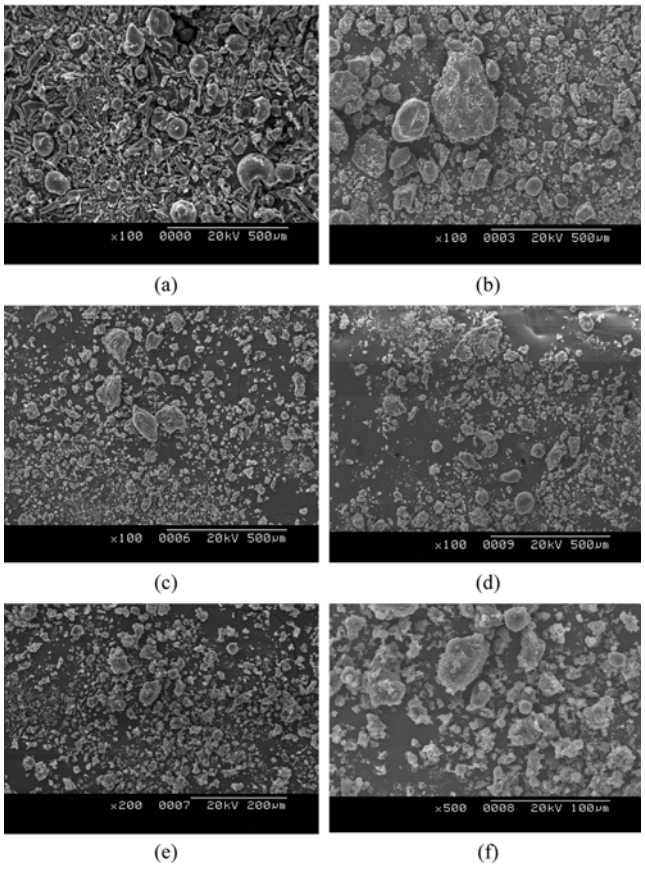

Fig. 3. SEM images of the particles obtained by PGSS using SC-CO_2. (a) Original PEG; (b) 20 MPa, 40 °C, (c) 20 MPa, 50 °C and (d) 25 MPa, 50 °C with the nozzle size of 300 μm; (e) 25 MPa, 50 °C and (f) 20 MPa, 50 °C with the nozzle size of 250 μm.

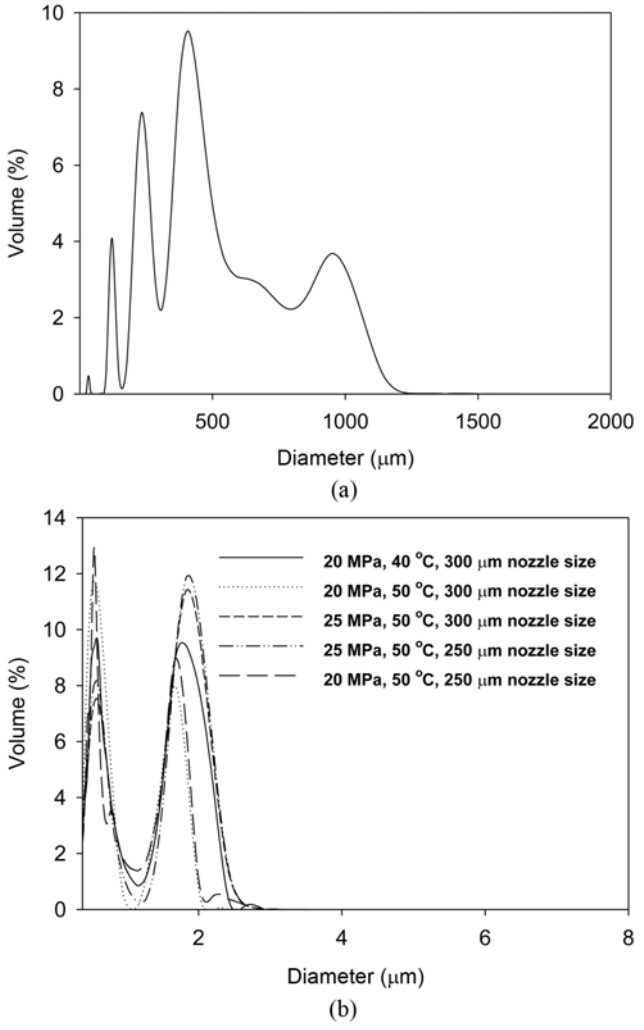

Fig. 4. Particle size and distribution volume of (a) original PEG and (b) particle formed by PGSS.

in Tables 3 and 4. The average size of particles was found to range from 0.78 to 1.42. Particle size was remarkably affected by temperatures, pressures, stirring speed and nozzle sizes. The lowest average size of particle was found at 30 MPa and 40 °C with the stirring speed of 400 rpm and nozzle size of 250 μm. The particle size decreased with the increasing of stirring speed in both nozzles used. It can be explained by the fact that higher stirring speed and smaller nozzle size may make super-saturation of particles. A significant effect of temperature on the particle size was observed. The particle size increased with the increase in temperature. It happened due to decrease of solvating power of SC-CO_2 with increase in temperature. On the other hand, the decrease of temperature and the increase of pressure raised the solvating power of SC-CO_2. It might be explained that CO_2 could not escape easily due to rapid solidification of the particles due to the temperature effect on particle formation. Nalawade et al. [1] also reported the similar effect of temperature on micro particle formation by PGSS using SC-CO_2. In contrast, at constant temperature the particle size of functional compounds with PEG decreased with the increase in pressure. It might have happened due to increase of solvating power of SC-CO_2. This result also agreed with the increase of particle size with increasing temperature. Nozzle size also moderately affected the size of particles with PEG. In most cases, the particle size obtained by PGSS using 300 μm was bigger than that of obtained by PGSS using 250 μm.

3. Quantitative Measurement of Functional Pigment by HPLC Analysis

The amount of functional pigment contained by PEG in PGSS process is shown in Tables 1 and 2. At various conditions, the amount of

Table 1. The amount of astaxanthin and fucoxanthin in the particles formed by PGSS using SC-CO_2 at 40 °C

Pressure (MPa)	Stirring speed (rpm)	Astaxanthin (mg/g of particle formed with PEG)		Fucoxanthin (μg/g of particle formed with PEG)	
		Nozzle (250 μm)	Nozzle (300 μm)	Nozzle (250 μm)	Nozzle (300 μm)
10	200	15.8	15.9	0.000457	0.000481
	300	15.7	16.2	0.000477	0.000486
	400	15.8	16.4	0.000475	0.000491
15	200	18.0	17.8	0.000499	0.000498
	300	17.8	18.1	0.000498	0.000502
	400	18.2	17.9	0.000508	0.000511
20	200	20.1	19.6	0.000527	0.000532
	300	20.2	19.8	0.000531	0.000537
	400	20.5	20.1	0.000539	0.000541
25	200	21.6	21.4	0.000683	0.000685
	300	21.8	21.8	0.000689	0.000688
	400	22.1	22.0	0.000695	0.000691
30	200	22.1	22.5	0.000716	0.000732
	300	22.4	22.6	0.000719	0.000736
	400	22.3	22.9	0.000725	0.000737

Table 2. The amount of zz and fucoxanthin in the particles formed by PGSS using SC-CO_2 at 50 °C

Pressure (bar)	Stirring speed (RPM)	Astaxanthin (mg/g of particle formed with PEG)		Fucoxanthin (μg/g of particle formed with PEG)	
		Nozzle (250 μm)	Nozzle (300 μm)	Nozzle (250 μm)	Nozzle (250 μm)
10	200	15.8	16.2	0.000645	0.000678
	300	15.9	16.4	0.000665	0.000682
	400	16.2	16.7	0.000687	0.000681
15	200	18.1	18.6	0.000674	0.000689
	300	18.5	18.8	0.000684	0.000691
	400	18.4	19.0	0.000682	0.000695
20	200	20.6	21.2	0.000699	0.000712
	300	20.7	21.4	0.000703	0.000711
	400	21.0	21.7	0.000721	0.000718
25	200	25.1	26.1	0.000938	0.000954
	300	25.5	26.0	0.000945	0.000965
	400	25.8	26.3	0.000943	0.000958
30	200	26.4	28.2	0.000957	0.001024
	300	26.6	28.0	0.000959	0.001023
	400	26.9	28.6	0.000968	0.001029

Table 3. The average diameter of the particles obtained by PGSS using SC-CO_2 at 40 °C

Pressure (MPa)	Stiring speed (rpm)	Average diameter (μm)	
		Nozzle size (250 μm)	Nozzle size (300 μm)
10	200	1.24	1.34
	300	1.18	1.36
	400	1.16	1.27
15	200	1.12	1.29
	300	1.24	1.24
	400	1.07	1.22
20	200	0.98	1.25
	300	1.01	1.19
	400	0.92	1.16
25	200	0.84	1.28
	300	0.79	1.24
	400	0.81	1.25
30	200	0.81	1.25
	300	0.84	1.21
	400	0.78	1.19

Table 4. The average diameter of the particles obtained by PGSS using SC-CO_2 at 50 °C

Pressure (MPa)	Stirring speed (rpm)	Average diameter (μm)	
		Nozzle size (250 μm)	Nozzle size (300 μm)
10	200	1.42	1.53
	300	1.43	1.56
	400	1.37	1.48
15	200	1.34	1.48
	300	1.29	1.47
	400	1.27	1.51
20	200	1.32	1.39
	300	1.34	1.34
	400	1.27	1.27
25	200	1.12	1.31
	300	1.09	1.32
	400	1.02	1.27
30	200	1.08	1.27
	300	1.11	1.19
	400	0.97	1.11

included fucoxanthin in PEG was ranging from 0.000457 to 0.001029 μg/g of dried sample and astaxanthin was ranging from 15.7 to 28.6 mg/g of standard sample. It was found that higher amounts of fucoxanthin and astaxanthin were included in PEG at 30 MPa and 50 °C with stirring speed of 400 rpm and nozzle size of 300 μm. At high pressure and temperature, the solubility of pigments and PEG was higher and that enhanced the inclusion of functional compounds in PEG by PGSS process. The solubility of pigments and PEG depends on a complex balance between the increase in the SC-CO_2 density and increase in vapor pressure of pigment. Some researchers reported similar effects of pigment solubility in SC-CO_2 from different sources [20,25,26]. The amount of included astaxanthin in PEG was much higher than that of fucoxanthin in PEG. It might have happened due to the presence of impurities in the extracted amount of fucoxanthin in PGSS process compared to the amount of astaxanthin standard used.

CONCLUSIONS

Particles of functional pigment with PEG were formed by PGSS using SC-CO_2. Most of the particles prepared by PGSS were found to be spherical with average diameter of 1.18 μm. The particles obtained at different conditions showed a considerable size reduction with a uniform size distribution volume, and itwas due to the unique physical properties of supercritical fluids. The different pressure and temperature on the particle size distribution obtained by PGSS had moderate effect, but influenced the amount of the active compounds in PEG mixtures in which higher pressure and temperature caused the increase in solvating power of CO_2. Further studies should be considered to explore the time release and the stability of the active compounds in particles prepared by PGSS process with various conditions.

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