

Preparation of Microparticles of Functional Pigments by Gas-Saturated Solutions 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. 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 improving product preservation as well as controlling the dissolution rate of the active compound. The particles from gas-saturated solution (PGSS) process has been used to micronize suspensions of different substances in polymer melts, in order to obtain composite materials. The particle formation of functional pigment with biodegradable polymer, polyethelene glycol (PEG) was performed by supercritical carbon dioxide (SCO₂) in a thermostated stirred vessel. PGSS were carried out in different temperatures and pressures to measure the optimum condition for the formation of functional pigment particle (pressure from 20 MPa to 25 MPa, temperature from 40 to 50°C). The average diameter of the particles obtained at different conditions was about 0.8-1.4 μm accompanied by the size reduction, comparing the size of unprocessed particles, 430 μm.

INTRODUCTION

Application of supercritical fluids has significantly increased in the past decade. The properties of supercritical fluids can be tuned by changing the fluid density between those of liquid and gases, have been adopted or are being explored as extraction, fractionation, chromatography, crystallization, polymerization and particle formation. Particle formation using SCO₂ was important for drug delivery systems and will most likely be the next major commercial application area [1]. The bioavailability, the ratio of drug absorbed in target area by body to initial dosage, can be improved by decreasing the particle size and maximize the surface area, which leads to an increasing of dissolution. Small particles of pharmaceuticals with a narrow particle size distribution plays a vital role in the design of conventional drug delivery systems like tablet, capsule, injection; biphasic drug delivery system like suspension and emulsion and the controlled drug delivery systems of implants, transdermal, microemulsions, nanoparticulate [2-6]. The particle formation technology that uses supercritical fluids has devolved in many types during the last 20 years. Traditionally, some methods are in use for producing fine particles, including milling, grinding, spray drying, recrystallization from solution. But almost particles using these method have drawbacks such as the degradation of the product in the mechanical treatments because high temperature and toxic solvents. Applications of supercritical fluids may overcome the problem of conventional processes [7-10].

PEG is one of the commonly used compounds in 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 form for pharmaceutical applications, such as drug carriers [11]. The functional compounds, fucoxanthin and astaxanthin have anti cancer effect [12-13]. Brown sea weed (*Undaria pinnatifida*) contains carotenoids in which fucoxanthin is the principal pigment. Therefore, the aim of this study was to make fine particles of functional pigment, fucoxanthin from brown seaweed and standard astaxanthin.

MATERIALS AND METHODS

Materials

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

Sample preparation

Brown seaweeds were dried in an freeze dryer. The dried sample was mechanically crushed and sieved in 500 μm by mesh. The sieved sample was used for SCO_2 extraction of oil.

SCO_2 extraction

Brown seaweed sample was extraction by SCO_2 for fucoxanthin extraction. The set up of a laboratory scale supercritical fluid extraction process is shown in Fig.1. Brown seaweed sample (12 g) was loaded into the 50ml stainless steel extractor. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with 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 were measured using a gas flow meter. The oil extracted by SCO_2 was collected by a glass separating vessel. The temperature and pressure for SCO_2 extraction were 45°C and 20 MPa.

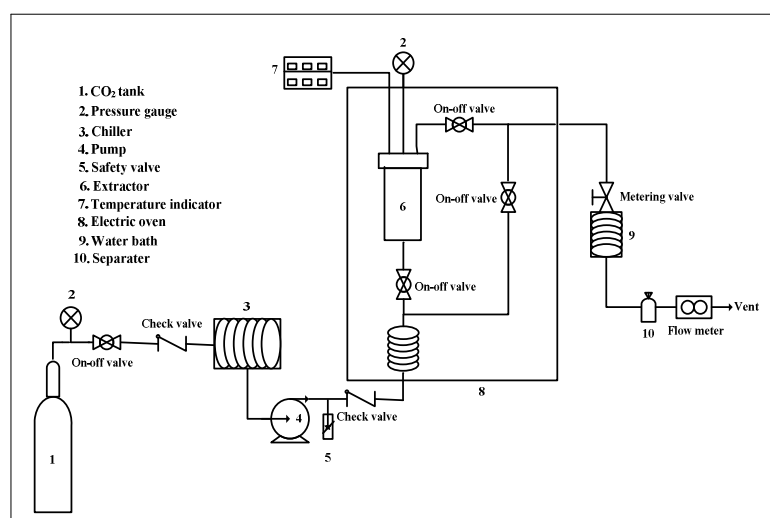


Figure 1: Flow diagram of the supercritical fluid extraction system

HPLC analysis for functional pigment

Fucoxanthin and astaxanthin measurement was carried out using a Waters HPLC equipped with a model 600E system controller, a model 484 UV/VIS detector and a Eclipse Plus C18 column (5 μ m, 4.6 x 250 mm, Agilent, USA). 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) were used for fucoxanthin and astaxanthin, respectively. Both mobile phases were eluted 1 mL/min as a isocratic method. Fucoxanthin and astaxanthin was detected at the wavelength of 449 and 486 nm, respectively. The solvents were filtered through a 0.25 μ m advantec filter prior use and degassed by sonicator. The injection volume and flow-rate were 20 μ L. The amount of fucoxanthin/astaxanthin in was measured based on the peak area of the standard fucoxanthin/astaxanthin.

Particle formation

The PGSS process is shown in Fig.2. A PGSS experiment began by delivering SCO₂ to the precipitation chamber until the desired pressure was reached. Polyethylene glycol and pigment samples (standard astaxanthin and extracted oil) (5.7 g / 0.3 g) in reactor were melted by SCO₂ and mixed by stirred heel. These experimental conditions were ranged from temperature 40 to 50°C and pressure from 10 MPa to 30 MPa. The mixture was stirred at 400 rpm and the nozzle size was 250/300 μ m. The duration for reactions were one hr. After reaction, functional pigment with SCO₂ delivered through nozzle and collected in a separator.

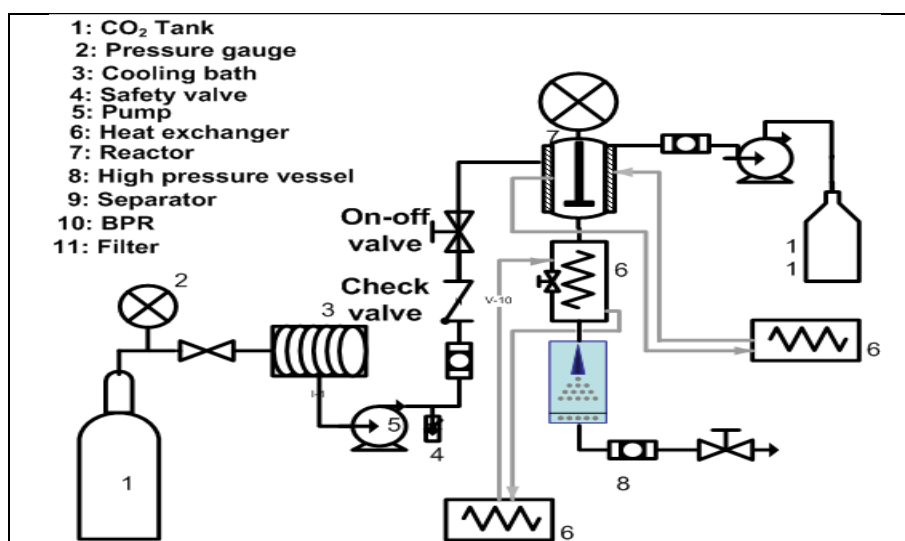


Figure 2: Schematic diagram of PGSS process

Scanning electron microscope analysis

Samples of the powder precipitated on the metallic frit were observed by a scanning electron microscope (SEM). The SEM samples were covered with 250 Å of gold using a sputter coater. Particle sizes were measured from SEM images using the Sigma Scan Pro image analysis software.

RESULTS

Extraction of oil from Brown Seaweed

The oil extracted by SCO_2 from brown sea weed at 20 MPa and 45°C was 0.53 g/12 g brown sea weed (data not shown). The increase of the extraction yield was not significant over 50 min extraction time.

Particle formation

This process is designed for making particles of materials that absorb supercritical fluids at high concentrations. Several experiments have performed at different temperatures in supercritical states using two different nozzles, 250 and 300 μm , for PEG 8000. SEM image results of powder generation from PEG 8000 using different pressure and temperature are shown in Figure 3. These results can be explained in detail with the help of the various parameters such as the temperature, pressure.

Some experiments have been carried out below the melting point of the polymer; it is requisite to ensure that PEG 8000 is present in the liquid state under these conditions. It has already been reported in literature that the dissolved CO_2 in PEG causes a considerable decrease in the melting point.

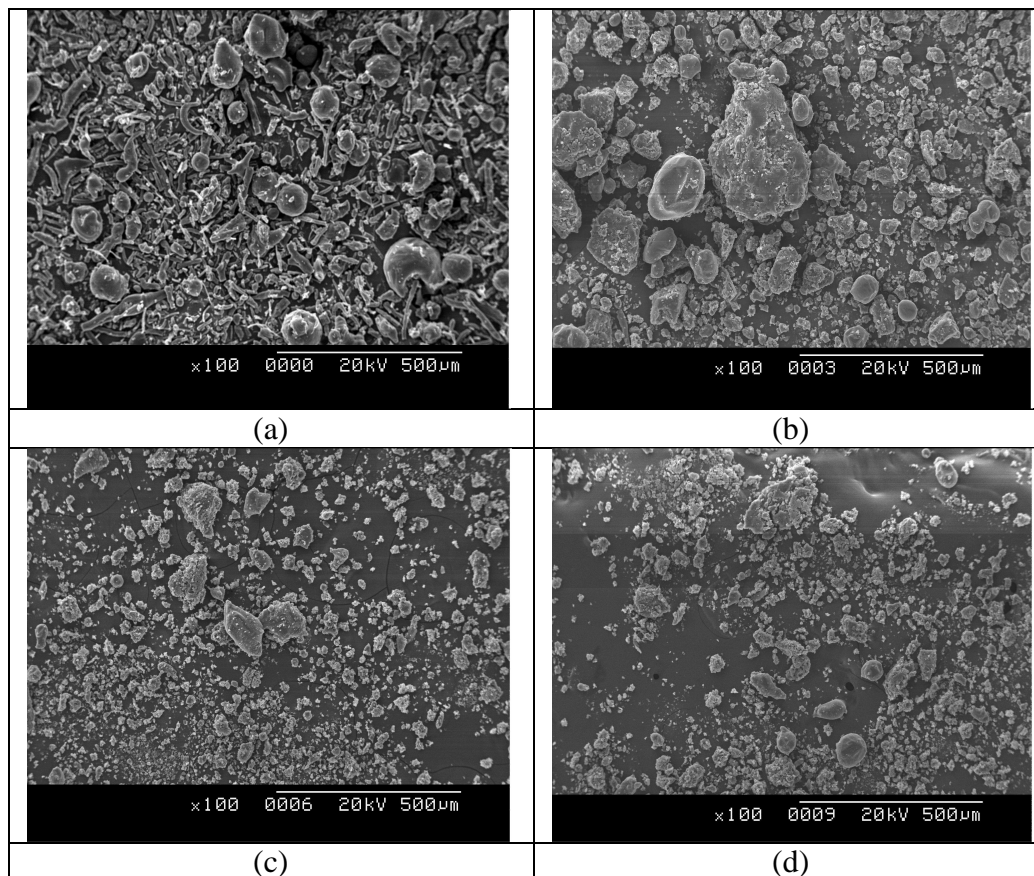


Figure 3: SEM pictures of the particles obtained using a 300 μm nozzle at: (a) Before treated PEG; (b) 20 MPa, 40°C; (c) 20 MPa, 50°C; (d) 25 Mpa, 50°C

Quantitative measurement of functional pigment by HPLC analysis

Table 1 shows functional pigment quantity contained by PEG. The amount of included fucoxanthin was ranging from 0.000527 to 0.000954 $\mu\text{g/g}$ in dried sample and astaxanthin was ranging from 187 to 261 mg/g in standard by various conditions. In this study, the amounts of fucoxanthin and astaxanthin included by PEG were much higher than other experiment condition at 25 MPa, 50°C and 300 μm nozzle. When the pressure was high the solubility of pigments and PEG were also high. The yield depends on a complex balance between the increase in the SCO_2 density, increase in vapor pressure of pigment as the temperature and according to nozzle size increase blast pressure. This represented the solubility of the pigment in the SCO_2 .

Table 1: The amount of astaxanthin and fucoxanthin in the particles formed by PEG and SCO_2 at different conditions

Nozzle size (μm)	Temp. ($^{\circ}\text{C}$)	Pressure (MPa)	Astaxanthin (mg/g)	Fucoxanthin ($\mu\text{g/g}$)
300	50	20	212	0.000712
		25	261	0.000954
	40	20	196	0.000532
		25	214	0.000675
250	50	20	206	0.000699
		25	251	0.000938
	40	20	187	0.000527
		25	201	0.000683

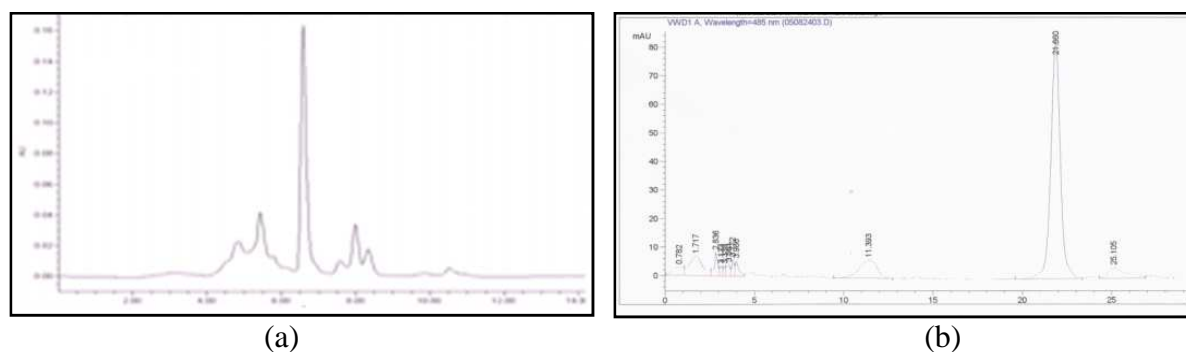


Figure 4: HPLC analysis of active compounds in the particles prepared by PGSS: (a) Fucoxanthin, (b) Astaxanthin

Particle size analysis (PSA)

Figure 5 shows the particle size distribution of the particles of PEG 8000 obtained under different conditions. Particle size was affected by temperature, pressure and nozzle size. Lowest particle size was found at 25 MPa, 50°C, 300 μm nozzle size. The effect of changing pressure on the particle size and shape was found up to 3 μm . The average particle size of PEG before treatment was almost 400 times higher than that of PEG obtained by SCO_2 treatment. All experimental results of powder generation from PEG 8000 using different size nozzle are shown in Table 2.

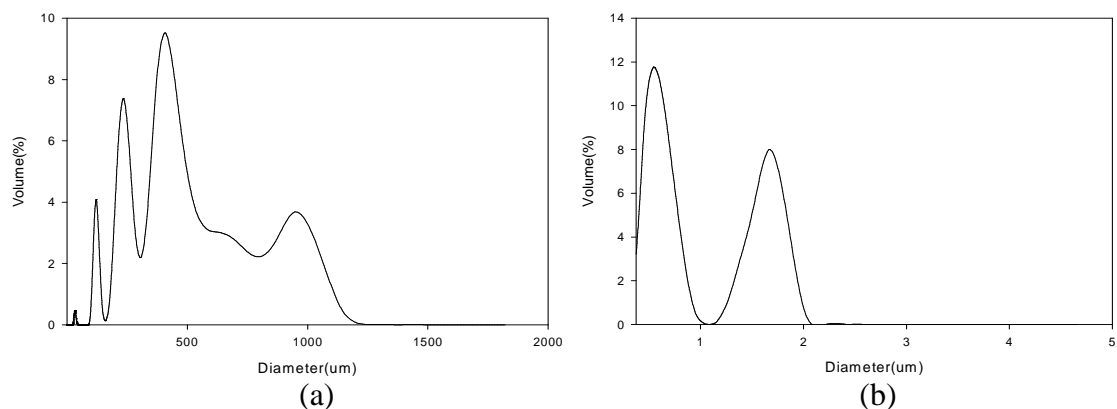


Figure 5: Particle size and distribution of the PEG(a) and the sample(b) obtained conditions, 250 μm nozzle at 25 MPa and 50°C

Table 2: The average diameter of the particles obtained from different conditions

Nozzle size (μm)	Temp. ($^{\circ}\text{C}$)	Pressure (MPa)	Average diameter (μm)
300	50	20	1.25
		25	1.28
	40	20	1.39
		25	1.31
250	50	20	0.98
		25	0.84
	40	20	1.32
		25	1.12

CONCLUSION

In this study we could show that by the PGSS process functional pigment included microparticles could be produced. Characterization of particles prepared in this study showed a spherical morphology with average diameter about 1.18 μm . Also the particles obtained at different conditions showed a considerable size reduction with a uniformed size distribution. These results are derived due to the unique physical properties of supercritical fluids. The effect of pressure and temperature on the particle size distribution have been found not significant at supercritical fluid conditions, but influenced the amount of the active compounds in PEG mixtures which higher pressure and temperature cause the increasing of the solubility of CO_2 . Further studies are necessary in order to explore the time release and the stability of active compounds in particles prepared by PGSS process with various conditions.

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