Effect of solvent on nanoparticle production of β-carotene by a supercritical anti-solvent process

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ABSTRACT

Production of micro- to nano-sized particles of β-carotene was investigated using the solution-enhanced dispersion by supercritical fluids (SEDS) process. β-Carotene was dissolved in dichloromethane (DCM), *n,n*-dimethylformamide (DMF), *n*-hexane, or ethyl acetate, and supercritical CO_2 was used as an anti-solvent. The effects of the organic solvent, operating pressure, and temperature were examined. The morphologies of the particles produced by the SEDS were observed by field emission-scanning electron microscopy and particle sizes were determined by image analysis. Irregularly shaped micro particles were produced in the system which uses DCM and DMF solution. Plate-like micro particles were produced by using *n*-hexane solution, and irregular nanoparticles were produced by using ethyl acetate solution. The optimum operating conditions were determined to be ethyl acetate as solvent operated at 8–12 MPa and 40–60 °C.

Keywords: β-Carotene; Nanoparticle formation; Supercritical anti-solvent, Supercritical CO_2

1. INTRODUCTION

Carotenoids pigments, found in plants and some other photosynthetic organisms, consist of eight isoprene units joined in a head-to-tail pattern, and have highly vivid colours. The main function of carotenoids in plants is the removal of active oxygen generated by various bioreactions [1]. Carotenoids are widely used as high-quality natural colorants in the food, cosmetic, and nutraceutical industries [2]. It is also used in dietary supplements because it exhibits many functionalities such as an inhibitory effect for several types of cancer, including lung and mammary cancers and leukaemia, in addition to antioxidant activities [3–5]. Industrial carotenoids are usually crystalline powders that are soluble in oils and organic solvents, and they have low water solubilities [6,7]. β-Carotene is one of the most common carotenoid pigments used in the industries. The physicochemical properties of β-carotene are shown in **Table 1** [8]. Micronized β-carotene particles are more effective for human nutrition, since micronization increases the β-carotene concentration in the serum and lymph. The miniaturization of carotenoid particles may enhance the bioavailability, without causing excessive their accumulation in the body [9].

Fine particle formation using supercritical carbon dioxide (SC-CO_2) has recently attracted attention. Since CO_2 has low critical temperature, it may be suitable for heat-sensitive materials. Additionally, SC-CO_2 can be easily separated from the produced particles along with the organic solvent as SC-CO_2 again becomes the gas phase at ambient temperature and pressure. The supercritical anti-solvent (SAS) process is one of the micronization methods that can be applied for making fine particles from various materials such as pharmaceutical and cosmetic materials and pigments [10–15]. In a typical SAS process, a solution containing compounds to be processed and an organic solvent are fed via a nozzle into a precipitator to produce particles filled with SC-CO_2. Supersaturation was caused by mixing of the solution with SC-CO_2 in the precipitator, leading to crystal nucleation and growth. Several factors affect the particle size and morphology, such as operating temperature, pressure, CO_2 and solution flow rate, kind of organic solvent, and the nozzle type and its inner diameter [15–23].

The solution-enhanced dispersion by supercritical fluids (SEDS) process is one of the modified SAS processes. In this process, the solution and SC-CO_2 are sprayed into a precipitator by a coaxial nozzle [11,24–28]

Water-soluble nanoparticles of the carotenoid/cyclodextrin complex were produced for synthesizing medicines by the SEDS process [29]. In this study, the organic solvent used was *n,n*-dimethylformamide (DMF), which is not approved anywhere in the world for use in food or supplement products. In this work, fine particle production of β-carotene with some organic solvents that are approved as food additives has been investigated for application to food products. The most appropriate solvent was selected from *n*-hexane, ethyl acetate, dichloromethane (DCM), and DMF. The effects of operating pressure and temperature of the process on the size and shape of the obtained particles were examined. The SEDS process was carried out in a semi-continuous cell at 8–12 MPa and 40–60 °C. The morphology of the particles generated was observed by field emission-scanning electron microscopy (FE-SEM).

2. MATERIALS AND METHODS

2.1 Materials and chemicals

β-Carotene (purity >80%), which was crushed mechanically, was purchased from Wako Pure Chemical Industries, Ltd., Japan. DCM (purity >99%), DMF (purity >99.5%), *n*-hexane (purity >95%), and ethyl acetate (purity 99.3%) were obtained from Kanto Chemical Co., Inc., Japan. CO_2 (purity >99.5%) was supplied by Sogo Co., Japan.

2.2 Equipment and procedures

The SEDS process was carried out in a semi-continuous precipitation vessel. **Figure 1** shows a schematic diagram of the SEDS apparatus. The apparatus consists of a CO_2 chiller (Cooling Unit CLU-33, Iwaki Asahi Techno Glass, Tokyo, Japan), HPLC pumps for CO_2 and solution (PU-980 Intelligent HPLC pump, JASCO Co., Tokyo, Japan), a heating chamber (Incubator EI-700B, AS ONE Co, Osaka, Japan), a precipitation vessel (SUS316 cell, inner diameter: 3 cm, length: 17 cm, volume: 120 cm^3, maximum design pressure: 30 MPa), a coaxial nozzle (**Fig. 2**, nozzle inner diameters: 2.4 and 0.8 mm, inner diameter of the nozzle outlet: 0.4 mm, custom-made by Taiatsu Techno Co., Tokyo, Japan), a wet gas meter for CO_2 flow rate (Sinagawa Co., Tokyo, Japan), a membrane filter for collecting particles (100 nm PTFE membrane filter, Advantec, Tokyo, Japan) placed inside a Swagelok filter housing, and a back-pressure regulator (AKICO Co., Tokyo, Japan).

Experiments on the SEDS process were carried out as follows: liquefied CO_2 was introduced into the system using an HPLC pump (-5 °C and operating pressure) at a constant flow rate. The CO_2 was heated in the preheater, placed in heating chamber, to change it to the supercritical state. When the temperature and pressure in the system reached the desired operating conditions, β-carotene dissolved in the organic solvent (1.5 mg/mL) was pumped using an HPLC pump (at the operating temperature and pressure) at the desired flow rate. SC-CO_2 and the β-carotene solution were mixed and introduced into the precipitator via the coaxial nozzle. β-Carotene was precipitated by supersaturation by using the anti-solvent effect. After stopping the solution feed pump, the particles and the system line were washed with SC-CO_2 to help remove the organic solvent from the particles. Finally, particles were collected from the membrane filter after depressurization. The experiments were carried out at operating pressures 8–12 MPa and operating temperatures 40–60 °C. The concentration of β-carotene in the organic solvent (DCM, DMF, *n*-hexane, and ethyl acetate) was 1.5 mg/mL. The flow rates of the solution and supercritical CO_2 were 0.25 and 20 mL/min (at the pump conditions), respectively. **Table 2** shows the detailed experimental conditions.

2.3 Analysis and characterization

The shape and surface characteristics of the raw materials and the SEDS-processed particles were observed using a FE-SEM (S-5200, Hitachi, Tokyo, Japan). The samples were sputter-coated with gold in a high-vacuum evaporator and examined using FE-SEM at 30 kV. The particle sizes and size distributions were measured using Image J software which was developed by the National Institutes of Health.

3. RESULTS AND DISCUSSION

3.1 Selection of solvent

The suitability of the solvent in the SEDS process was tested at 12 MPa, 40 °C and concentration of 1.5 mg/mL for solvents, DCM, DMF, *n*-hexane, and ethyl acetate. **Table 3** shows the physicochemical properties of these organic solvents from Chemical database by National Center for Manufacturing Sciences, USA and Showa Chemical Industry Co.,Ltd., Japan. The experiments showed that particles were obtained for all conditions; however, their size and morphology were different, depending on the solvent. **Figure 3** shows the SEM image of the raw material for β-carotene, which was crushed mechanically. **Figure 4** shows the SEM image of the particles produced by SEDS. The particle size of the raw material for β-carotene was 2–15 μm. In the case of processing with DCM and DMF solution, irregular micro-particles were formed. On the other hand, plate-like micro-particles were generated by using *n*-hexane as the solvent. Irregular nanoparticles were precipitated by ethyl acetate solution, as shown in Fig. 4. The critical point of the binary system can be found from the phase equilibrium curve. **Figure 5** shows the phase compositions for the systems of CO_2 and DMF, DCM, *n*-hexane, and ethyl acetate, respectively, at 40 or 45 °C [30–33]. The maximum value of the four curves indicates a critical point of the mixture of organic solvent and CO_2. The critical point of the CO_2+DMF system is at 9 MPa, while for all the other solvents it is near 8 MPa.

As seen in Fig. 5, for the pressure of 4 MPa condition, the compositions of the CO_2 and organic solvent systems were different for each type of solvent. The most soluble organic solvent was ethyl acetate, followed in the decreasing order by hexane, DCM, and DMF. The solubility is an important parameter in this process. The particle size is reduced by increasing the supersaturation levels. The supersaturation depends on the difference of β-carotene solubility between before and after the contact of the organic solvent with SC-CO_2.

The characteristics of subcritical CO_2, such as density, viscosity, diffusivity, and surface tension are quite different in the SC-CO_2 region [34]. The reason why the size and shape of the particles changed, depending on the kind of organic solvent used, is considered next. The effects of the SAS system on the solubility of the three-component system comprising β-carotene, CO_2, and organic solvent should be evaluated. However, because there is no data available on this three-component system and the calculation process would be highly complicated, this system has been discussed by using solubility parameters [37-38] and experimentally obtained solubility data of β-carotene in various organic solvents [37]. **Table 4** shows the Hansen solubility parameters of β-carotene and organic solvents. Additionally, Hansen solubility parameters of CO_2 at various temperature and pressure conditions were calculated by King; for the 40 °C and 12 MPa condition, the total solubility parameter of CO_2 was about 10 [38]. Hansen solubility parameters are used to predict the solubility of a substance based on the following concept: two substances are easily soluble with each other when the molecules of the respective substances exhibit similar interactions. Hansen solubility parameters consist of three parameters, δ_d, δ_p, and δ_h, which denote the dispersion, polar, and hydrogen bonding interactions, respectively. When the parameters of two components are located near each other in the Hansen space, it means that the two molecules will easily dissolve into each other.

The total Hansen solubility parameter can be calculated using Eq. (1)

$$(\delta_t)^2 = (\delta_d)^2 + (\delta_p)^2 + (\delta_h)^2 \qquad (1)$$

Where δ_t is the total solubility parameter, δ_d is the dispersion solubility parameter, δ_p is the polar solubility parameter, and δ_h is the hydrogen bonding solubility parameter [35].

The SEDS process is related to both equilibria and transport phenomena. Although transport phenomena are important factor which influences the particle morphology, we focus on the equilibria factor to discuss the effect of solvent. The total solubility parameter, δ_t of ethyl acetate is closest to that of β-carotene among the solvent used in this work, although tendency of solubility of β-carotene in organic solvent is different from that of the solubility parameter as shown in **Table 5**. In SEDS process, the solubility of the carotenoid in organic solvent is very important and also complicated parameter. When the solubility of carotenoid in the solvent is too high, crystal generation is suppressed because of reduced anti-solvent power. When organic solvent has a certain level of solubility of carotenoid, crystal generation and growth occur easily by contact with the SC-CO_2 as anti-solvent. Therefore, it is desirable to use an organic solvent which have suitable balance of the solubility for both SC-CO_2 and carotenoids. In the case of ethyl acetate system, smaller uniform particles were obtained. Consequently, it is considered that ethyl acetate has the most suitable solubility balance with SC-CO_2 and β-carotene compared to the other solvents such as DCM, DMF and n-hexane. The morphology of the β-carotene particles was also quite different, depending on the organic solvent, probably because polymorphic crystals are affected by the solvent in which they were dissolved [39].

3.2 Effect of pressure and temperature

The effects of pressure and temperature on the morphology and size of the treated particles were studied at constant concentrations of β-carotene in the ethyl acetate solution. The effect of pressure was considered in the pressure range 8–12 MPa and at a constant temperature of 40 °C. The particle sizes decrease with increasing pressure as shown in **Fig. 6**. This trend has also been observed in a previous work [29]. When the process was carried out at a lower pressure, the particles tended to be larger and plate-like. However, as the pressure increased, irregularly shaped nanoparticles were obtained. The smallest particles (diameter of around 135 nm) were formed at 10-12 MPa and 40 °C. This decrease in the particle size with increasing pressure can be explained by the rate of SC-CO_2 diffusion into the organic solvent droplets that is dependent on the operation pressure [40]. The solubility of the solvent in SC-CO_2 increases at higher pressures [41–43]. Moreover, the droplet size will also decrease with an increase in the SC-CO_2 density, and reduces the interfacial tension between SC-CO_2 and the solvent at higher pressures. This leads to SC-CO_2 diffusing into the solvent immediately when the pressure increases. The partial molar volume and cohesive energy density of the organic solvent will decrease due to an increase in the diffusive driving force. Therefore, the higher supersaturation of the carotenoid in the solvent is caused by a higher mass transfer rate and a higher solubility of the solvent in SC-CO_2. Therefore, the solvent power of the organic solvent for the solute decreases rapidly, causing the particle to become smaller [40].

The experiments were also carried out at various temperatures: 40, 50, and 60 °C, at a pressure of 12 MPa. Particle size increased with increasing temperature as shown in **Fig. 7**. The smallest particles, with a diameter of around 135 nm, were formed at 40 °C and 12 MPa. Higher temperatures reduced the CO_2 density, forming larger droplets. Therefore, a lower

CO_2 density leads to a higher solvent power for the solute, and it causes a lower mass transfer rate and lower supersaturation of solute in the organic solvent [40]. This explains why the particles sizes were reduced at lower temperatures. Therefore, the smallest particles of β-carotene were formed at above the critical pressure (10–12 MPa) and at the lowest temperature (40 °C), because the ethyl acetate solution immediately became supersaturated under these conditions [16,31,44].

4. CONCLUSION

Nano-particles of β-carotene were successfully produced by the SEDS process. The effects of parameters such as the kind of organic solvent, operating pressure, and temperature on the particle morphology and size were investigated. Irregularly formed micro-particles (5 to 20 μm) were precipitated when processing with DCM and DMF solution. Plate-like micro-particles (10 to 20 μm) were generated by using *n*-hexane as the solvent. Irregularly formed nanoparticles (135 nm) were precipitate by using ethyl acetate solution. The reduction of β-carotene particle size from 10 μm to 135 nm by increasing pressure was observed. And particles size of β-carotene were changed from 135 nm to 10 mm by increasing temperature. Optimum conditions for fine particle production (135 nm mean size) were found this work to be 12 MPa and 40 °C and using ethyl acetate as organic solvent.

ACKNOWLEDGMENTS

This research is funded by the Japan Society for the Promotion of Science (JSPS) and Japan Science and Technology Agency (JST).

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Tables with headings

Table 1. Physicochemical properties of β-carotene (Source: Skeget et al., 1997)

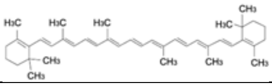
	Formula	Molecular weight	Melting point (°C)	Critical point
β-Carotene	$C_{40}H_{56}$ 	536.87	172–173	1212.42 °C 799.3 MPa

Table 2. Experimental conditions (Concentration of 1.5 mg/mL, liquefied CO_2 flow rate of 20 mL/min, solution flow rate of 0.25mL/min)

Sample No.	Solvent	Pressure (MPa)	Temperature (°C)
1	*n*-Hexane	12	40
2	Ethyl acetate	12	40
3	DCM	12	40
4	DMF	12	40
5	Ethyl acetate	8	40
6	Ethyl acetate	10	40
7	Ethyl acetate	12	50
8	Ethyl acetate	12	60

DCM; dichloromethane, DMF; *n,n*-dimethylformamide

Table 3. Physicochemical properties of organic solvents (Source: Chemical database by NCMS and Showa Chemical Co., Ltd)

	Formula	Molecular weight (g/mol)	Melting point (°C)	Boiling point (°C)
n-Hexane	C_6H_{14}	86.18	-95	69
Ethyl acetate	$C_4H_8O_2$	88.11	-83.6	77
DCM	CH_2Cl_2	84.93	-96.7	39.8
DMF	C_3H_7NO	73.09	-61	153

DCM: dichloromethane, DMF: *n,n*-dimethylformamide.

Table 4. Hansen solubility parameters of β-carotene and organic solvents

	Solubility parameter ($MPa^{1/2}$)			
	δ_d	δ_p	δ_h	δ_t
β-Carotene (25 °C)	17.1	2.39	5.54	18.0
n-Hexane (RT)	14.9	0	0	14.9
Ethyl acetate (RT)	15.8	5.3	7.2	18.1
DCM (RT)	18.2	6.3	6.1	20.2
DMF (RT)	17.4	13.7	11.3	24.9

δ_d: Dispersion solubility parameter, δ_p: Polar solubility parameter, δ_h: Hydrogen bonding solubility parameter, δ_t : Total solubility parameter, RT: Room temperature, DCM: dichloromethane, DMF: *n,n*-dimethylformamide

Table 5. Solubility of β-carotene in organic solvents (Source: Craft et al., 1992)

Organic solvent	Solubility [mg/L]
n-Hexane	600
Ethyl acetate	500
DCM	6000
DMF	200

DCM: dichloromethane, DMF: *n,n*-dimethylformamide

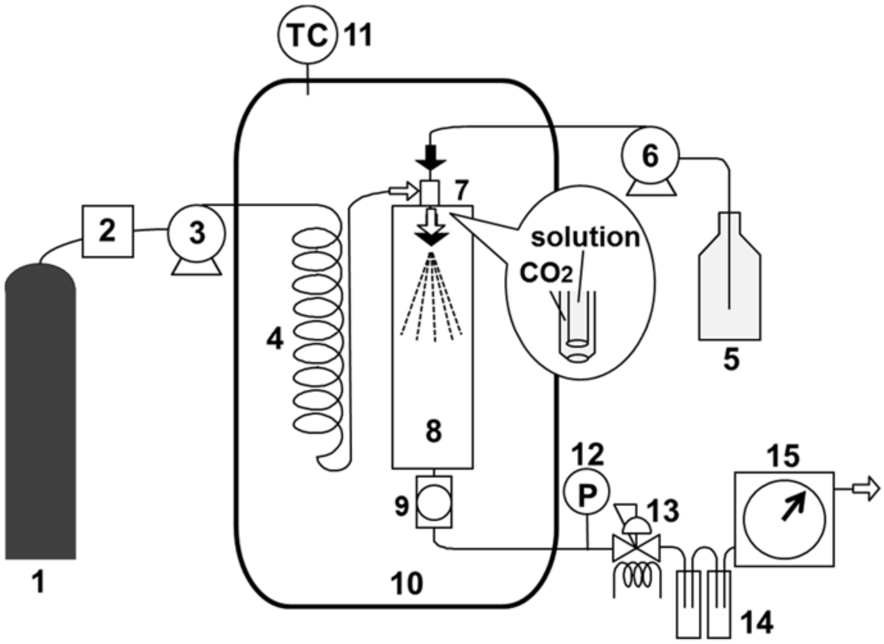


Figure. 1: Schematic diagram of the SEDS process. (1) CO_2 cylinder, (2) chiller, (3) CO_2 pump, (4) CO_2 pre-heater, (5) carotenoid solution, (6) feed pump, (7) coaxial nozzle, (8) precipitator, (9) membrane filter placed in Swagelok filter, (10) heating chamber, (11) temperature controller, (12) pressure gauge, (13) back-pressure regulator, (14) trap, and (15) wet gas meter. ([1]SEDS: Solution enhanced dispersion by supercritical fluids)

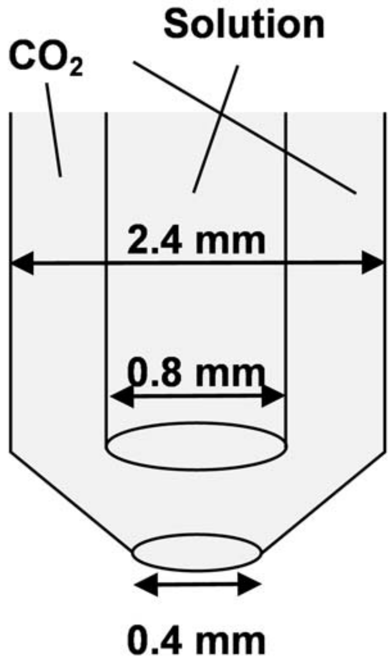


Figure. 2: Schematic diagram of the coaxial nozzle.

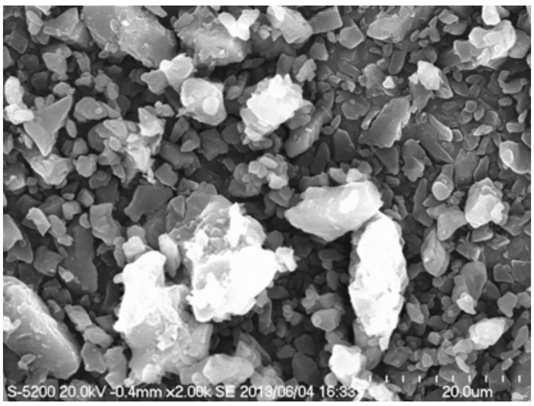


Figure. 3: SEM image of the raw material for the β-carotene crystal.

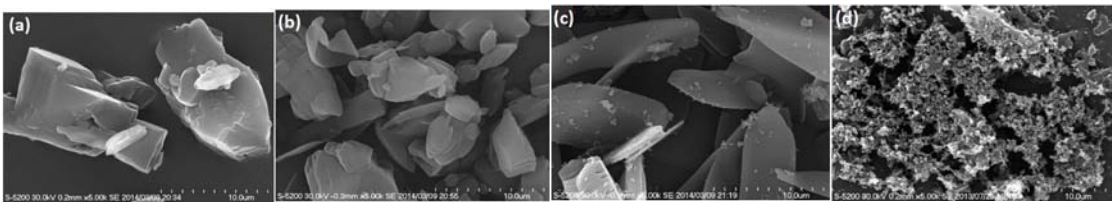


Figure. 4: SEM image of β-carotene particles treated with various solvents ((a) dichloromethane, (b) *N,N*-dimethylformamide, (c) *n*-hexane and (d) ethyl acetate) under the following conditions: temperature, 40 °C; pressure, 12 MPa; lCO_2 flow rate, 20 mL/min; solution flow rate, 0.25 mL/min; and concentration, 1.5 mg/m.

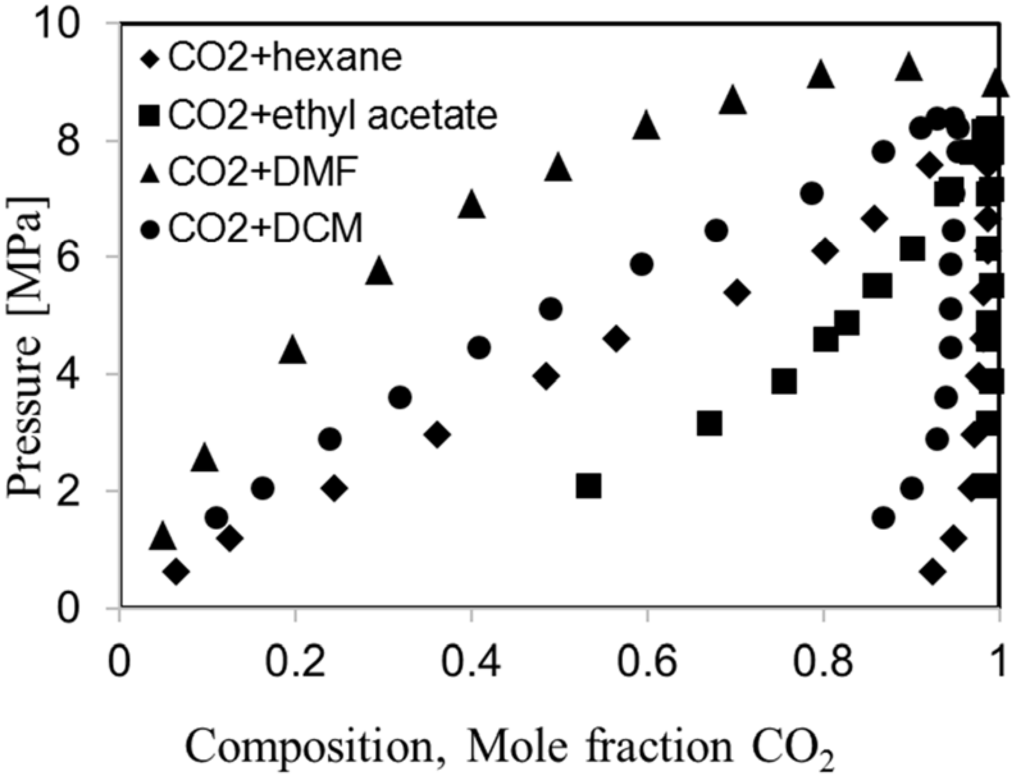


Figure. 5: Phase composition for the system of CO_2 with *N,N*-dimethylformamide (DMF) (40 °C), dichloromethane (DCM) (45 °C), *n*-hexane (40 °C), and ethyl acetate (40 °C), using reported experimental data (Ohgaki et al., 1976; Wagner et al., 1994; Temtem et al., 2006; Tsivintzelis et al., 2004).

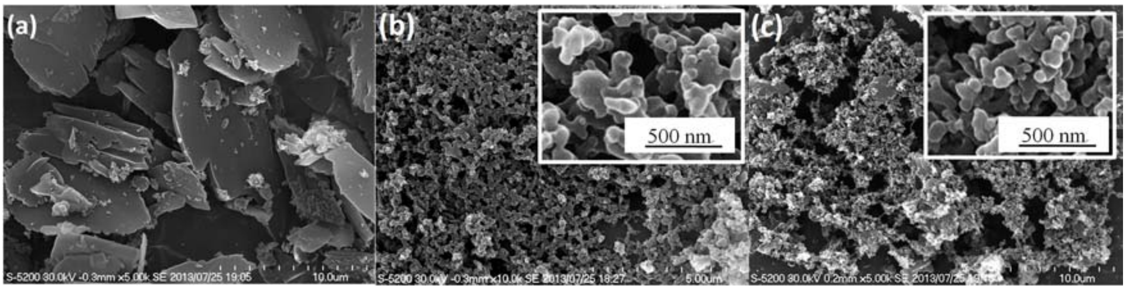


Figure. 6: SEM image of treated particles precipitated from an ethyl acetate solution of β-carotene (1.5 mg/mL) under the following conditions: temperature, 40 °C; CO_2 flow rate, 20 mL/min; solution flow rate, 0.5 mL/min; and various pressures ((a) 8 MPa, (b) 10 MPa and (c)12 MPa).

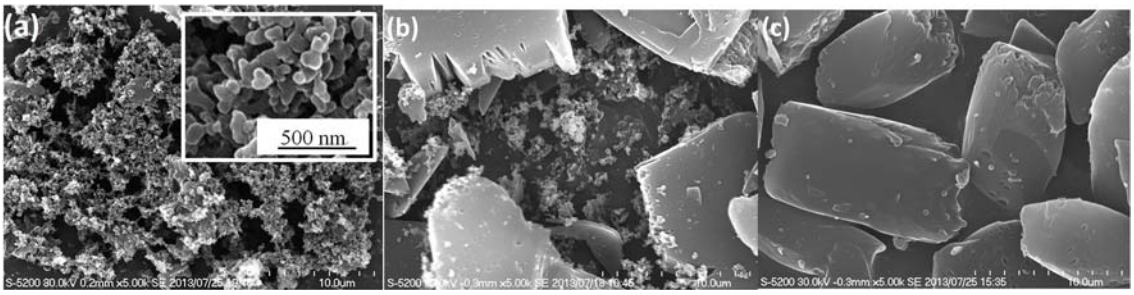


Figure. 7: SEM image of treated particles precipitated from an ethyl acetate solution of β-carotene (1.5 mg/mL) under the following conditions: pressure, 12 MPa; CO_2 flow rate, 20 mL/min; solution flow rate, 0.5 mL/min; and various temperatures ((a) 40 °C, (b) 50 °C and (c) 60 °C).

Type of Article: Table of Contents text.

・Micro- to nano-sized particles of beta-carotene were produced by SEDS.
・Ethyl acetate was a more suitable solvent than DCM, DMF, and hexane for the process.
・Nano-particles were obtained with an ethyl acetate solution at 40 °C and 10-12 MPa.

Title

Effect of solvent on nanoparticle production of β-carotene by a supercritical anti-solvent process

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Chem. Eng. Technol. **20XX**, *XX (X)*,
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