Particle Formation of Lecithin Process with Particles from Gas-Saturated Solutions using Supercritical Carbon Dioxide

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ABSTRACT

The particles from gas-saturated solutions process was performed to micronize lecithin isolated by ethanol from deoiled mackerel by Supercritical Carbon Dioxide (SC-CO₂). The particle formation of lecithin with biodegradable polymer, polyethylene glycol (PEG) was carried out by PGSS process using SC-CO₂. Conditions were optimized by applying different temperatures (40 and 50°C) and pressures (15-30 MPa) for PGSS process for 1 hr. The particles were characterized by scanning electron microscope (SEM) and Particle size analyzer (PSA) to determine their shape. The average diameter of lecithin particle was high at higher temperature and pressure. The average diameter of the particles was about 0.37-2.42 μm. The inclusion of lecithin in PEG was qualitative measured by HPLC.

Keywords: particles; particles from gas-saturated solutions (PGSS); polyethylene glycol (PEG); supercritical carbon dioxide; lecithin

1. Introduction

The formulation of natural substances together with a biocompatible or biodegradable carrier material to form composites or encapsulates has a great relevance for pharmaceutical, cosmetic and food industries [1]. Natural substances such as carotenoids, fatty acids, natural antioxidants are being extensively used on a great variety of food products [2].

Lecithin is a sticky fatty substance composed mainly of phospholipid mixtures with small amount of glycerides, neutral lipids and other suspended matter. Pharmacological use of lecithin includes in treatments

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for hypercholesterolemia, neurologic disorders and liver ailments. Lecithin has also been used to modify the immune system by activating specific and nonspecific defence systems [3-5].

Pharmaceutical particle formations using SC-CO$_2$, such as RESS, PGSS, and SAS methods have received much attention as alternative precipitation methods to those with organic solvents [6]. Particle formation using SC-CO$_2$ is important for drug delivery systems that 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 [1].

In this study, lecithin has been isolated from deoiled mackerel obtained by SC-CO$_2$ extraction and continuous process for the formation of particle with PEG by PGSS process.

2. Materials and Methods

2.1. Materials

Mackerel were purchased from fish market. Removed viscera, and was dried in a freeze-drier for about 72 h. The dried sample was crushed by mechanical blender and sieved 700 $\mu$m by mesh. These dried samples were then stored at -20°C until using for SC-CO$_2$ extraction. Pure CO$_2$ (99.99%) was supplied by KOSEM, Korea. Standard of PC were purchased from Sigma-Aldrich, USA. All reagents used in this study were of analytical or HPLC grade.

2.2. SC-CO$_2$ Extraction

A laboratory-scale supercritical fluid extraction process was used to oil extract from mackerel. The flow rate of CO$_2$ (22 g/min) was constant over the entire extraction period of 2.5 h, and extraction parameters were different temperatures (35-45°C) and pressures (15-25 MPa). Mackerel residues providing the highest oil yield by SC-CO$_2$ extraction were used for lecithin isolation.

2.3. Isolation of lecithin

Lecithin was isolated from deoiled mackerel obtained by SC-CO$_2$ extraction according to the method of Wang [9] with modifications. Briefly, 100 mL of ethanol (95%) was added to 30 g of SC-CO$_2$ extracted mackerel residues and stirred for almost 24 h by a magnetic stirrer. The mixture was then centrifuged at 1900 g for 10 min. The supernatant containing mainly polar lipids was collected and evaporated in a vacuum rotary evaporator.

2.4. Particle formation

The experiments were carried out using PEG 8000 and lecithin with different pressures and melting temperatures. The schematic diagram of PGSS process used in this study is shown in Fig. 1. Lecithin and PEG (1:10) in reactor were melted by SC-CO$_2$ and mixed by stirred heel. These experiments were carried out at temperatures, 40 to 50°C and pressures, 15 to 30 MPa. The mixture was stirred at 250 rpm and the nozzle size was 300 $\mu$m. The duration for reactions was 1 h. After reaction, lecithin with PEG were sprayed through nozzle and collected in a separator.

Fig. 1. Schematic diagram of PGSS process.
2.5. *Analysis of particle by scanning electron microscope (SEM) and Particle size analyzer (PSA)*

Samples of the PEG with lecithin on the metallic frit were observed by a scanning electron microscope. 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.

The size distribution of the PEG was measured by particle size analyzer (PSA). The result from the analysis is the relative distribution of volume of particles in the range of size classes.

2.6. *Lecithin qualitative analysis in PEG by HPLC*

In this study, major phospholipids, Phosphatidyl Choline (PC) at PEG particle were qualitative by a Jasco HPLC equipment with an evaporative light scattering detector (ELSD-Softa corporation, Model 400) and a silica column (5 μm, 4.6 mm x 250 mm, Waters, USA). The analysis was carried out according to the method of *Letter* [7] with modification of the ELSD operation.

3. *Results and Discussion*

3.1. *Characterization of encapsulation particle obtained by PGSS process*

3.1.1. *Analysis of SEM images*

The particles produced by PGSS process were characterized by SEM. Fig. 2. shows SEM images of micro particles of lecithin with PEG obtained by SC-CO₂ treatment at different pressures and temperatures. Almost all particles size was decreased by different temperatures and pressures but PEG shape was not spherical shape. *Cocero et al.* [1] reported that the initial concentrations of active substance are key parameters with respect to the morphology of the particles. Lecithin is sticky, and it had emulsification ability [10], that effect particles aggregation.

![Fig. 2. SEM images of the particles obtained by PGSS process using SC-CO₂. (a) Original PEG: (b) 50oC, 15 MPa; (c) 40 oC, 30 MPa and (d) 50°C 30 MPa with the nozzle size of 300 μm.](image)

3.1.2. *Particle size analysis by PSA*

The size distributions of original PEG and lecithin particles with PEG obtained by PGSS process using SC-CO₂ under different conditions are shown in Fig. 3. In this study, the largest particle size was found to be 2.4 μm. The average sizes of lecithin particles with PEG obtained by PGSS process at different conditions are shown in Fig. 3 (b) and (c). The average size of particles was found to be ranged from 0.37 to 2.4 μm at
nozzle size of 300 μm. On the other hand, the average size of original PEG was 36.23 to 282.05 μm. The average particle size of PEG before PGSS process was almost 100 times bigger than that of PEG obtained by PGSS process using SC-CO₂.

Fig. 3. SEM images of the particles obtained by PGSS process using SC-CO₂. (a) Original PEG; (b) 50°C, 15 MPa; (c) 40°C, 30 MPa and (d) 50°C, 30 MPa with the nozzle size of 300 μm.

3.2. Qualitative analysis of lecithin

The major phospholipids of lecithin were PC and Phosphatidyl Ethanolamine (PE). Cho et al. [8] and other study reported that phospholipids from squid and egg-yolk contained 71.7% and 80.5% of PC [9]. In this study, major phospholipids of lecithin, PC was qualitifid by HPLC and that shows under Fig. 4.

Fig. 4. A chromatogram of PC by HPLC-ELSD (a) PC standards and (b) particle formed by PGSS at 20 MPa and 40°C. Second point
4. Conclusion

In this study, the particles of lecithin with PEG were formed by PGSS using SC-CO$_2$ at different conditions. The particles formed by PGSS process at different conditions showed a considerable size reduction with a uniformed size distribution volume and it was due to the unique physical properties of supercritical fluids. Major phospholipid of lecithin, with PEG was PC. The qualitative phospholipid was performed based on the peak area of standard phospholipid, PC. Further studies should be considered to measure the quantification of lecithin with PEG by HPLC and the stability of active compounds in particles prepared by PGSS process with various conditions.

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References


