

Micro-flow Process of Emulsification and Supercritical Fluid Extraction of Emulsion for Stearic Acid Lipid Nanoparticle Production

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Abstract

In this study, emulsification and supercritical fluid extraction of emulsion are combined in micro-flow process for the formation of stearic acid lipid nanoparticles dispersed in aqueous solution. Controllability of lipid particles formation by surfactant species, surfactant concentrations and pressure in the flow system are investigated. The utilization of two different organic surfactant species is considered for the stabilization of oil droplets and lipid nanoparticles in water. Tween 80 as hydrophilic surfactant has an important role in homogeneous emulsion formation which is necessary to control particle size after the extraction process. Lecithin as hydrophobic surfactant can be applied in order to enhance the uniformity of lipid nanoparticle size distribution. Extraction performance in the flow system is acceptable with low residual organic solvent in the product of nanoparticle dispersion. Low pressure condition is preferable to avoid aggregated form of nanoparticles occurred in high operating pressure.

Keywords

supercritical carbon dioxide, solid lipid nanoparticle, microfluidic system

1. Introduction

Solid lipid nanoparticles (SLN) represent as an active ingredients carrier that gives the attractive advantages such as controlled release and degradation protection of active compounds [1]. Lipid nanoparticles are highly acceptable to use in food, cosmetic and pharmaceutical products among other types of nanoparticles due to their non-toxicity property [2]. However,

controllability of produced SLN have to be carefully considered from various formation techniques for the effective applications.

Traditionally, nanoparticles can be fabricated by using solvent evaporation from oil in water emulsion [3, 4]. High temperature is required to remove organic solvent which results in precipitation of nanoparticles. However, the high temperature condition causes the degradation of active compounds. Besides, the remained amount of organic solvent is a matter of considerable concern for this fabrication technique. The fabrication methods with mild processing temperature and high extraction performance are preferable for nanoparticles production.

Supercritical fluid extraction of emulsion (SFEE) has been applied for fabrication of SLN. Utilization of supercritical carbon dioxide (scCO_2) can remove organic solvent at the mild processing temperature which overcomes the degradation problem in conventional evaporation method. However, the large vessels or columns used in the conventional SFEE are considered as the obstacles for scaling up and consuming high cost and time of the operation. These problems are suggested to be overcome by the introduction of microfluidic system for SFEE which allows the reduction of costs and time of the operation [5].

In this study, emulsification by micro-swirl mixer and SFEE in microchannel (inner diameter of 500 μm) are combined in micro-flow system for the formation of stearic acid lipid nanoparticles dispersed in aqueous solution. The understanding of phenomenon about emulsification and scCO_2 extraction in the flow system with the utilization of combined surfactant species and variation of pressure are necessary for the controllability of SLN production in the practical application.

The objective of this work is to investigate the effect of the surfactant species, surfactant concentrations and pressure using in the micro-flow process of emulsification and SFEE on the size of stearic acid nanoparticles and extraction efficiency of ethyl acetate (EA, organic solvent) from the ethyl acetate in water (EA/w) emulsion. Two types of organic surfactants used in this study are Tween 80 as hydrophilic surfactant and lecithin as hydrophobic surfactant. Tween 80 and lecithin were dissolved in water phase and EA phase, respectively. The prepared raw materials of both water phase and EA phase were fed and mixed to form the emulsion at the micro-swirl mixer instead of using vessel in conventional process. This micro mixer would reduce mixing units when the process is scaled up to the large production. The flow of emulsion was continuously mixed with scCO_2 for the extraction in microchannel instead of using large column. The extraction in microchannel can reduce cost and time of the high pressure operation. The liquid outlet product from the extraction process was collected and considered as SLN dispersion. The size distribution of oil droplet in emulsion and SLN in dispersion samples were analyzed by dynamic light scattering (DLS) method. The concentration of residual EA in the dispersion products were analyzed by HPLC system. The results from the experiment were

considered in term of surface stabilization from organic surfactants and the phenomenon during different pressure conditions.

2. Experimental

2.1. Materials

Ultra-pure water was produced by using Direct-Q UV3 Water Purification System supplied by EMD Millipore Corporation, Japan. Ethyl acetate (purity > 99.5%), stearic acid (purity > 95.0%), lecithin from egg (phospholipid > 95.0%), polyoxyethylene (20) sorbitan monooleate (Tween80), methanol (purity > 99.8%) and acetic acid (purity >99.9%) were supplied from Wako Pure Chem. Ind., Japan. Carbon dioxide with the purity over 99.95% was supplied by Fujii Bussan Co., Ltd., Japan.

2.2. Apparatus and Procedure

Tween80 aqueous solution (0 to 3.0 wt.%) was prepared by stirring for 1 h at the room temperature. Stearic acid (0.5 wt.%) and lecithin (0 to 1.5 wt.%) were dissolved into EA. The schematic diagram of emulsification and SFEE in micro-flow process is given in Fig. 1. Prepared oil phase and water phase were separately supplied to the mixing part at the first micro-swirl mixer by HPLC pumps (PU-4180 from JASCO, Japan). Inner diameter of microchannel used in the process is 500 μm . The feeding flow ratio between oil phase and water phase was 1:4. The sample of emulsion was collected from separated output of emulsion line for the analysis.

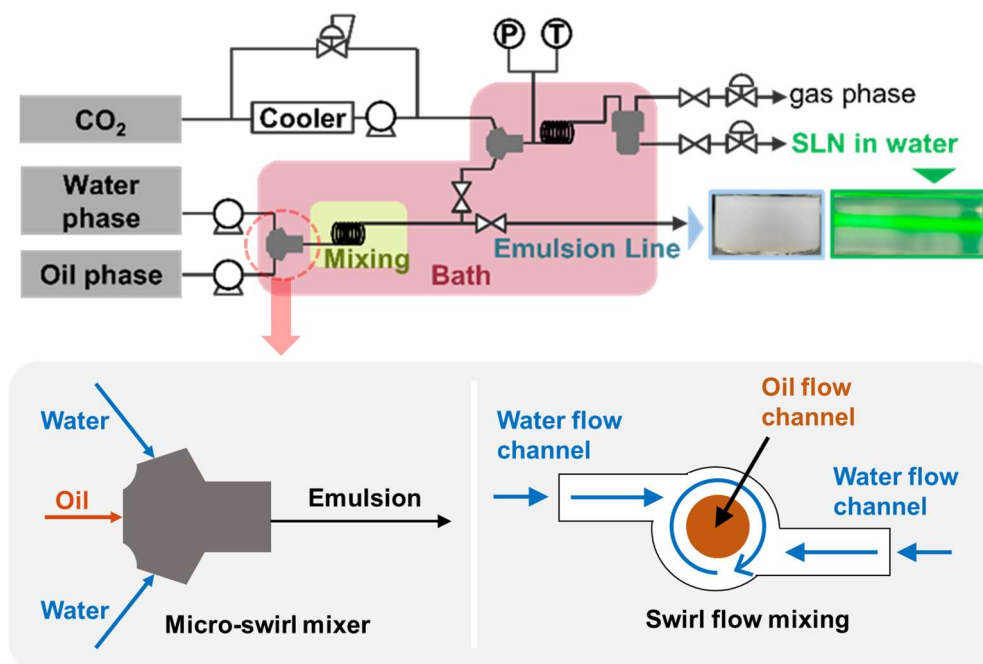


Fig. 1. Schematic diagram of emulsification and SFEE in micro-flow process.

For SLN production, CO₂ from the gas cylinder was delivered through a silica gel column for dehydration and cooled down to form liquified CO₂. The liquified CO₂ was pressurized by a semi-preparative CO₂ pump (PU-4386 from JASCO, Japan). The pressurized CO₂ was then heated in a pre-heating coil sunk in a thermostat water bath at 313.15 K. Supercritical carbon dioxide and emulsion were continuously flowed to be mixed by the second micro-swirl mixer. The pressure of micro-flow system was regulated by a back pressure regulator. Pressure and temperature were measured by a pressure gauge and thermometer which installed after the second micro-mixer. The EA extraction using scCO₂ proceeded in microchannel with the inner diameter of 500 μm . The scCO₂ phase included with EA was separated from the dispersion to the upper part of high-pressure vessel and flowed out from the system. The CO₂ flow rate was controlled by a needle valve set at the exit of the process. The extracted organic solvent in scCO₂ was trapped by water after depressurization. The flow rate of CO₂ was measured by a dry gas flow meter (GF 1010 from GL Science, Japan) and controlled in the range between 500 and 600 ml min⁻¹. The particle dispersion obtained after SFEE in the microchannel was accumulated in the high-pressure vessel and flowed out through the liquid outlet stream of the process. The liquid phase included with SLN was then collected and analyzed.

The size of oil droplets in EA/w emulsion and SLN dispersion collected from the micro-flow process were measured by DLS method using nano Partica SZ-100-Z, HORIBA Ltd., Japan. The dispersion was contained in the glass cell for the analysis. An excitation laser with the wavelength of 532 nm was used for the measurements. The average size (\bar{D}) of droplet or SLN and standard deviation (SD) of three times measurement for droplet or particle size (D_i) of each sample analyzed by DLS method were calculated by equations (1) and (2), respectively.

$$\bar{D} = \frac{\sum_{i=1}^3 D_i}{3} \quad (1)$$

$$SD = \sqrt{\frac{\sum_{i=1}^3 (D_i - \bar{D})^2}{3}} \quad (2)$$

The morphology of SLN was visualized by transmission electron microscopy (H-7650 TEM, Hitachi Ltd., Japan). The accelerating voltage for TEM analysis was specified at 100 kV. A drop of SLN dispersion was placed on the 200-mesh copper microgrid (Nisshin EM Co., Ltd., Japan) to leave a thin film on the grid. The samples were analyzed and photographed at suitable magnification.

Remained EA was separated from the liquid product of SLN dispersion by using dialysis device (Float-A-Lyzer®G2, MWCO of 1000 kD, USA) over 8 h. The analysis of the concentration of the residual EA from dialysis method was conducted by using HPLC system

which supplied by JASCO, Japan. The HPLC system consists of column oven, HPLC pump, UV-vis detector and LC-Net II/ADC (Interface Box). The sample injection valve equipped with a 5 μ l loop. The column of HPLC used for the separation was Unifinepak C18 (3.0 mm ID \times 100 mm Length \times 3 μ m Particle size, JASCO, Japan). The mobile phase used for the analysis composed of 30 wt.% of methanol, 70 wt.% of ultra-pure water with 0.2 wt.% of acetic acid. The analysis condition was at 0.6 ml min⁻¹ of the mobile phase flow rate, 313.15 K of column oven temperature, 10 min of method time and 12 s of sample injection interval. The absorbance wavelength setting at UV-vis detector is 210 nm.

The extraction efficiency (*EE*) of EA was calculated by mass flow rate of EA feeding to the process ($Q_{in,EA}$) and the mass flow rate of EA in the water at the outlet stream as finalized in equation (3). The outlet flow rate of EA in the water was considered from mass fraction as the concentration of EA in water phase product ($C_{out,w,EA}$) from HPLC analysis and mass flow rate of water at the outlet stream ($Q_{out,w}$). Equality between the flow rates of water at the inlet and outlet of the system ($Q_{in,w} = Q_{out,w}$) is assumed because of extremely low amount of water extracted into scCO₂.

$$EE(\%) = \left(1 - \frac{C_{out,w,org} \times Q_{in,w}}{Q_{in,org}} \right) \times 100 \quad (3)$$

3. Results and discussion

3.1. Effect of Tween 80 as hydrophilic surfactant

The effect of concentration of Tween 80 was studied at the condition of 0.5 wt.% lecithin and pressure of 10.0 MPa. Homogeneity of emulsion proceeded by adding Tween 80, as shown in Fig. 2, suggesting the reduction of interfacial tension between EA and water. Therefore, Tween 80 has an important role to stabilize homogeneous phase of EA/w emulsion. However, bimodal distributions of SLN in water from the extraction using SFEE in micro-flow process are obtained in the cases of 0.5, 1.0 and 3.0 wt.% of Tween 80 concentration in water. From the bimodal distribution, the peak of small particle size is suggested as Tween 80 micelle. Tween 80 can escape from the surface of oil droplet to water phase during the extraction using scCO₂. The penetration of scCO₂ into the droplet results in destabilization of the droplets and leads to EA release causing in the extraction of EA. However, this destabilization also enhances the loss of Tween 80 in water due to its strong hydrophilicity property. In order to reduce the loss of Tween 80, lecithin as hydrophobic surfactant is utilized for the modification of surface stabilization property.

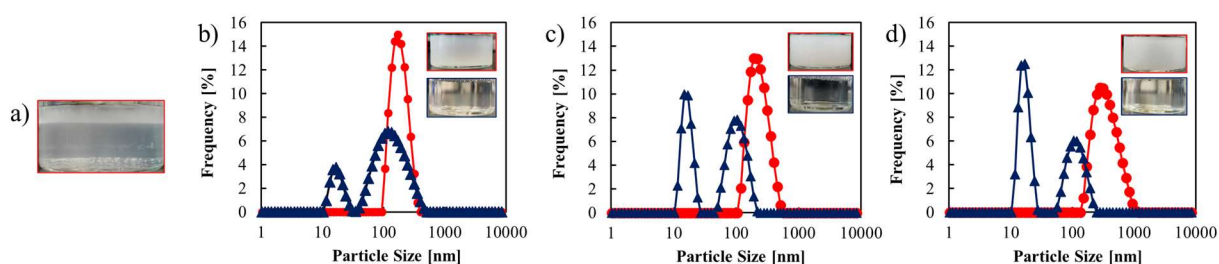


Fig. 2. Appearance and size distribution of ● EA/w emulsion and ▲ SLN dispersion for the Tween 80 concentration of a) 0, b) 0.5, c) 1.0 and d) 3.0 wt.% in water.

3.2. Effect of lecithin as hydrophobic surfactant

For the lecithin concentrations, the case of 1.0 wt.% Tween 80 aqueous at pressure of 10.0 MPa was considered. Uniform emulsion from emulsification in micro-flow process can be observed. As shown in Fig. 3, lecithin concentrations have no significant effect on the size of oil droplet. However, increasing amount of lecithin results in the variation of SLN size distribution from multimodal to unimodal. The solubility of lecithin in water is lower than Tween 80 which relates to the difficulty of surfactant loss from the surface during the extraction using scCO₂. Hydrophobic part of lecithin has stronger interaction with hydrophobic part of Tween 80 when compared to the interaction between Tween 80 molecules in the case of single component at the surface [6]. Therefore, the utilization of lecithin would assist the reduction of Tween 80 loss from the surface and enhance the stabilization of SLN in water.

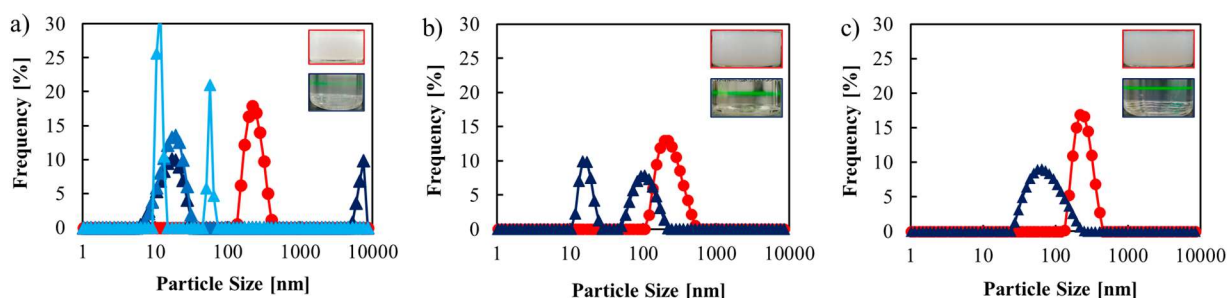


Fig. 3. Appearance and size distribution of ● EA/w emulsion and ▲ SLN dispersion for the Lecithin concentration of a) 0, b) 0.5 and c) 1.0 wt.% in EA.

3.3. Effect of operating pressure

The effect of pressure conditions from 8.5 to 20.0 MPa on fabrication of SLN dispersion was considered. Tween80 (1.0 wt.%) was dissolved in water. Stearic acid (0.5 wt.%) and lecithin (0.5 wt.%) were dissolved into EA.

Table 1 shows the effect of pressure conditions on SLN size and extraction efficiency of EA using scCO₂. The utilization of scCO₂ in micro-flow process of SFEE shows high extraction

efficiency over 97.0 % in all pressure conditions. The extraction using scCO₂ can minimize residual EA obtained in the product of dispersion to be lower than the limitation of 5000 ppm [7]. All pressure conditions are acceptable in the point of extraction performance.

However, the increment in pressure results the larger of particle size. This result can be explained by the loss of lecithin from the surface during the high pressure which reduce in stability and enhance the aggregation of the particles. The aggregation of particles was verified by TEM images of SLN dispersion samples from the cases of 8.5 and 20.0 MPa as shown in Fig. 4. The particles from SFEE in micro-flow system at 8.5 MPa shows the isolated form of SLN while 20.0 MPa shows the aggregated form. At high pressure, the density of scCO₂ increases which results in the increase of solubility of hydrophobic organic compounds in scCO₂. The lecithin molecules, hydrophobic surfactant, easily escape from the surface at high pressure condition which reduce the stability of the droplet and perform aggregation of SLN. Therefore, low pressure condition is preferable for the fabrication of stable SLN in water.

Table 1

Effect of pressure on average size of SLN, residual EA and *EE*.

Pressure (MPa)	Average size of SLN (nm)	Residual EA (ppm)	<i>EE</i> (%)
8.5	29.6 ± 0.96	2958.1	98.7
10.0	58.7 ± 1.91	3557.4	98.4
14.0	112.9 ± 6.16	4133.3	98.2
20.0	137.6 ± 11.31	4755.9	97.9

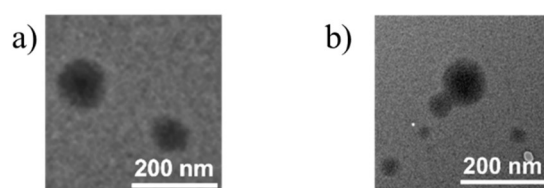


Fig. 4. TEM images of SLN in water from the pressure of a) 8.5 and b) 20.0 MPa.

4. Conclusion

The micro-flow process of emulsification and SFEE was successfully applied for the fabrication of SLN in water. Key importance of different organic surfactant species and operating pressure for SLN formation was investigated. Tween 80 as hydrophilic surfactant is necessary for the formation of homogeneous phase of EA/w emulsion. Lecithin as hydrophobic surfactant can be utilized for the fabrication of SLN with uniform particle size distribution. Combined surfactant species are preferable for fabrication of stable SLN in water. The operating

pressure from 8.5 to 20.0 MPa shows insignificant change of extraction efficiency of EA which is over 97.0 %. The high pressure condition at 20.0 MPa produced aggregated form of SLN which can be explained by the loss of lecithin from the surface. The low pressure is considered as a preferable condition to maintain the stability of SLN in water during the production.

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