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Microencapsulation and characterization of liposomal vesicles using a supercritical fluid process coupled with vacuum-driven cargo loading

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ABSTRACT

A new technique of liposomal microencapsulation, consisting of supercritical fluid extraction followed by rapid expansion of the supercritical solution and vacuum-driven cargo loading, was successfully developed. It is a continuous flow-through process without usage of any toxic organic solvent. For use as a coating material, the solubility of soy phospholipids in supercritical carbon dioxide was first determined using a dynamic equilibrium system and the data was correlated with the Chrastil model with good agreement. Liposomes were made with D-(+)-glucose as a cargo and their properties were characterized as functions of expansion pressure, temperature, and cargo loading rates. The highest encapsulation efficiency attained was 31.7% at the middle expansion pressure of 12.41 MPa, highest expansion temperature of 90 °C, and lowest cargo loading rate of 0.25 mL/s. The large unilamellar vesicles and multivesicular vesicles were observed to be a majority of the liposomes produced using this eco-friendly process.

1. Introduction

Liposomes are the microscopic spherical vesicles with one or more phospholipid bilayers separating an inner aqueous phase from the outer aqueous medium. They have long been used as models for studies on biological membranes due to their similarity to real cell membranes. The compound of interest is securely encapsulated in the inner aqueous phase surrounded by layered phospholipid membrane. Due to different size and lamellarity, there are several types of liposomal vesicles: small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), multilamellar vesicles (MLVs), and multivesicular vesicles (MVVs). The liposome size of SUVs varies from 20 to approximately 100 nm, whereas the sizes of LUVs, MLVs, and MVVs range from a few hundred nanometers to microns. The thickness of one phospholipid bilayer is reported to be 4 to 5 nm (Sharma & Sharma, 1997).

Liposomal microencapsulation has drawn great interest in the pharmaceutical industry (Torchilin, 2005). This technique can enhance the functionality of certain therapeutic compounds, such as the solubility improvement, controlled release, and targeted delivery (Kyrili et al., 2017; Liu et al., 2017; Nowald, Käsdorf, & Lieleg, 2017; Vahed, Salehi, Davaran, & Sharifi, 2017; Zhang et al., 2017). With the advance of this method, it has been applied in the food industry for microencapsulation of antioxidants, enzymes, and nutraceuticals (Ghorbanzade, Jafari, Akhavan, & Hadavi, 2017; Li, Liu, Han, Kong, & Liu, 2017; Manconi et al., 2016; Rafiee, Barzegar, Sahari, & Maherani, 2017; Tai et al., 2017). LUV is always preferred in the food industry due to its higher encapsulation efficiency, simpler process, and better stability (Gouin, 2004). Adding cholesterol, surfactants, or carbohydrate into the phospholipid bilayer can modify the rigidity, fluidity, and permeability of the liposomal membrane for specific purposes (Jo & Kim, 2009; Taylor, Davidson, Bruce, & Weiss, 2005; Yokoyama et al., 2005).

Traditionally, liposomes are prepared by thin film hydration (TFH), reverse phase evaporation (REV), and membrane extrusion. In the latest development, due to the concern of organic solvent toxicity, new techniques have been attempted to reduce or even completely avoid the use of organic solvents in the liposomal microencapsulation, including microfluidics, rapid expansion of a supercritical solution (RESS), supercritical reverse phase evaporation (scRPE), and several dense gas processes (Castor, 1996; Castor, 2005; Frederiksen, Anton, Hoogevest, Keller, & Leuenberger, 1997; Magnan, Badens, Commenges, & Charbit, 2000; Meure, Knott, Foster, & Dehghani, 2009; Otake, Shimomura, Goto, & Imura, 2006; Utada et al., 2005). In addition to being a green technology, the supercritical fluid (SCF) and dense gas methods require shorter operating time and less complicated steps with better quality control of the resultant liposomes, compared to the conventional processes for liposomal microencapsulation (Tsai & Rizvi, 2016a, 2016b).

When the pressure and temperature are raised above the critical point of a pure compound, the vaporization line ends, turning the coexisting gas and liquid phases into one homogenous phase, which is called a SCF. It exhibits some favorable properties, such as low viscosity, enhanced solvating power, tunable density, good diffusivity, and better

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mass transfer rate. A number of compounds, such as carbon dioxide, ethanol, nitrogen, and propane, have been used in their supercritical state. Carbon dioxide is the most preferable candidate due to its nontoxicity, eco-friendly attributes, and economical cost. Supercritical carbon dioxide (SC-CO_2) is a density-adjustable fluid with solvent behavior similar to hexane. Its moderate critical pressure (7.4 MPa) and low critical temperature (31.1 °C) make SC-CO_2 an ideal choice for biomaterial processing, including extraction of heat-labile compounds, high pressure pasteurization, and enzyme-catalyzed reactions (Kraujalis & Venskutonis, 2013; Wimmer & Zarevúcka, 2010; Yuk & Geveke, 2011). The SCF microencapsulation has been developed for two decades. However, some limits still exist in current SCF techniques, such as involvement of organic solvents, batch process, and low encapsulation efficiency, which may hinder their practicability for industrial applications.

Our objective was to develop an eco-friendly, efficient, and continuous process, consisting of SCF extraction followed by RESS and vacuum-driven cargo loading, for liposomal microencapsulation. It aimed to be a simple and rapid process for generation of the microencapsulated liposomes. SC-CO_2 was chosen as the sole phospholipid-dissolving medium without the use of organic solvents. Dynamic solubility of soy phospholipids (SPLs) in SC-CO_2 would be first measured to assure sufficient phospholipids dissolved in SC-CO_2. The optimal operating condition was to be applied for the pre-expansion part of this microencapsulation study. The vacuum, created at vena contracta in the expansion nozzle, would be tested for aqueous cargo loading without additional energy input. Liposome size, zeta potential, and encapsulation efficiency (EE) were to be characterized as functions of expansion pressure, temperature, and cargo loading rate to determine the optimized condition. Morphology of the liposomes was to be visualized using confocal laser scanning microscopy (CLSM). A semi-empirical model was then developed for impact evaluation of each operating parameter.

2. Materials and methods

2.1. Materials

Performix™ E soy lecithin was provided by Archer Daniels Midland Company (Chicago, IL, USA), containing 13% phosphatidylcholine (PC), 11% phosphatidylethanolamine (PE), and 8% phosphatidylinositol (PI), respectively. Cholesterol with 95% minimum purity, hexokinase assay kit, Nile Red, and fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris (hydroxymethyl) aminomethane (TRIS) was purchased from Bio-Rad (Hercules, CA, USA). D-(+)-glucose was purchased from Mallinckrodt Baker (Center Valley, PA, USA). Carbon dioxide with 99.99% minimum purity was purchased from Airgas (Ithaca, NY, USA).

2.2. Preparation of the fluorescent dyes and aqueous cargo

0.2 wt% of Nile Red in ethanol was prepared by adding 40 mg of Nile Red to 20 mL of absolute ethanol, followed by completely mixing using a vortex mixer. 5 mM FITC in pH 7.4 TRIS buffer was prepared as the aqueous cargo solution for phase-contrast imaging of the liposomes using CLSM. 0.2 M D-(+)-glucose solution in pH 7.4 TRIS buffer was prepared for this SCF microencapsulation.

2.3. Solubility measurements of SPLs in SC-CO_2

Dynamic solubility measurements of total SPLs in SC-CO_2 were conducted at three pressures (12.41, 16.55, 20.68 MPa) and three temperatures (60, 75, 90 °C). The operating condition for the highest phospholipids' solubility in SC-CO_2 would be applied to the pre-expansion of SCF liposomal microencapsulation.

The solubility measurements were adopted from our previous study (Tsai, Ruan, & Rizvi, 2006). The simplified schematic diagram of the SFT-250 SFE system (Supercritical Fluid Technologies, Newark, Delaware, USA) is shown in Fig. S1 in the Supplementary material. The system mainly consisted of a high pressure pump (HPP), extraction vessel (EV), and separation vessel (SV) for sample collection. The SC-CO_2 flow rate was controlled by a restrictor and metering valve (MV). 3 g of Performix E soy lecithin was loaded in the 100-mL EV and installed in the insulated chamber of the extraction unit. CO_2 was then introduced into the EV until the desired pressure and temperature was reached. After 30 min of phase equilibration, the MV was opened for the sample collection. The flow rate of SC-CO_2 was maintained at 0.67 g/min, where the saturated solubility of a solute in SC-CO_2 was ensured and observed to be independent of the flow-rate factor (Salgın & Salgın, 2013). After 2 h of SC-CO_2 extraction, the total amount of extracted phospholipids from soy lecithin were collected for analysis using a gravimetric method (Tejera-Garcia, Connell, Shaw, & Kinnunen, 2012). The solubility measurements of SPLs in SC-CO_2 were conducted in triplicates at each operating condition and reported as a mean ± standard deviation (SD) using Minitab 17 (Minitab Inc., State College, PA, USA).

2.4. SCF liposomal microencapsulation

The experiments were conducted at three expansion pressures (8.27, 12.41, and 16.55 MPa), three expansion temperatures (75, 83, and 90 °C), and two cargo loading rates (0.25 and 0.5 mL/s). The liposomes were characterized for their morphology, vesicle size, zeta potential, and encapsulation efficiency. The optimal operating condition aimed to be determined based on the highest encapsulation efficiency of D-(+)-glucose in the liposomes.

40 g of Performix E soy lecithin was well mixed with cholesterol in a mass ratio of 10:1. The purpose of adding cholesterol was to enhance the rigidity of liposomal membrane by changing interactions between both the polar head groups and hydrocarbon chains in the phospholipid bilayer, in order to avoid the collapse of liposomes immediately after SCF microencapsulation. A decrease in the melting point and transit enthalpy of the phospholipid/cholesterol mixture was also observed to assure well mixing of the cargo and lipids (Taylor et al., 2005). The lipid mixture was stored at 4 °C to be solidified for loading convenience.

The SCF microencapsulation system used for generation of the liposomes is shown in Fig. 1. The system mainly consisted of three parts: a high pressure pump (HPP), a mixing vessel, and an expansion nozzle. Different areas of the SCF system were heated by glass wool heating tapes and controlled by Variable Autotransformers (Type 3PN1010, 120 V for input and 0–120 V for output, Staco Energy Products Co., Dayton, OH, USA).

The solidified lipid mixture was loaded into the 2 L mixing vessel. CO_2 was then introduced by HPP into the vessel up to 20.68 ± 0.3 MPa, while the vessel temperature was maintained at 60 ± 1 °C. After at least 2 h of phase equilibrium, the phospholipids in soy lecithin were extracted with SC-CO_2. The lipid-laden SC-CO_2 was directed from the mixing vessel to expansion nozzle for liposomal microencapsulation, as shown in the blue solid arrows, and regulated by a metering valve (MV, Type SS-4L-MH, Swagelok, Western NY Fluid System Tech. Inc., Rochester, NY 14467). The expansion nozzle mixed the lipid-laden SC-CO_2 with the aqueous cargo in a 90-degree angle for optimal blending condition, based on our preliminary experiments.

The expansion pressure was regulated at 8.27, 12.41, and 16.55 MPa, respectively, using a forward pressure regulator (FPR, Model 44-1124-24-131, 68.94 MPa for maximum input and 17.24 MPa for maximum output, TESCOM, Elk River, MN, USA). Decreasing pressure from 20.68 MPa to the range between 16.55 and 8.27 MPa, when the lipid-laden SC-CO_2 flew from the mixing vessel to expansion nozzle, would assure that the saturated solubility of SPLs in SC-CO_2 was maintained while the operating temperature was varied through the system from 60 °C in the mixing vessel to the range of 75 and 90 °C in the nozzle. Therefore, sufficient phospholipids would be carried to the nozzle for