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Encapsulation of lutein in liposomes using supercritical carbon dioxide

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ABSTRACT

Liposomes loaded with lutein were prepared utilizing supercritical carbon dioxide (SC-CO_2). The effects of pressure, depressurization rate, temperature and lutein-to-lipid ratio on particle size distribution, zeta potential, encapsulation efficiency (EE), bioactive loading, morphology, phase transition and crystallinity were investigated. Liposomes prepared by the SC-CO_2 method had a particle size of 147.6 ± 1.9 nm–195.4 ± 2.3 nm, an encapsulation efficiency of 56.7 ± 0.7%–97.0 ± 0.8% and a zeta potential of −54.5 ± 1.2 mV to −61.7 ± 0.6 mV. A higher pressure (200–300 bar) and depressurization rate (90–200 bar/min) promoted a higher encapsulation of lutein whereas the lutein-to-lipid ratio had the dominant effect on the morphology of vesicles along with size distribution and EE. X-ray diffraction data implied a substantial drop in the crystallinity of lutein upon its redistribution in the liposome membranes. Differential scanning calorimetry indicated a broadened phase transition upon the simultaneous rearrangement of lutein and phospholipid molecules into liposomal vesicles. The SC-CO_2 method resulted in particle characteristics highly associated with the ability of CO_2 to disperse phospholipids and lutein molecules. It offers a promising approach to use dense phase CO_2 to homogenize hydrophobic or amphiphilic aggregates suspended in an aqueous medium and regulate the vesicular characteristics via pressure and depressurization rate. The SC-CO_2 method has potential for scalable production of liposomal nanovesicles with desirable characteristics and free of organic solvents.

1. Introduction

The development of novel encapsulation systems to deliver nutraceuticals, which may be sensitive to environmental conditions, has drawn growing attention (Diab, Jaafar-Maalej, Fessi, & Maincent, 2012). Liposomes have shown great promise as an encapsulation medium for the stabilization and delivery of nutraceuticals. As a self-assembled vesicle comprised of a bilayer of phospholipids enclosing an aqueous core, liposomes create a physical barrier to external unfavorable conditions and improve the bioavailability of encapsulated agents (Kraft, Freeling, Wang, & Ho, 2014). Unlike other nanocarriers, which may only encapsulate payload having a specific characteristic, liposomes facilitate accommodation of hydrophobic, hydrophilic and amphiphilic compounds due to the amphiphilic nature of phospholipids.

Liposomes have been studied as delivery vehicles to encapsulate curcumin (Takahashi, Uechi, Takara, Asikin, & Wada, 2009), resveratrol (Isailović et al., 2013), vitamins (Marsanasco, Márquez, Wagner, del V. Alonso, & Chiaramoni, 2011), quercetin (Liu et al., 2013) and others. Lutein is a plant-based carotenoid antioxidant that plays a vital role in maintaining eye function. Lutein works to prevent age-related macular degeneration (Olmedilla, Granado, Blanco, Vaquero, & Cajigal, 2001), cataract (Moeller et al., 2006), and retinitis pigmentosa (Berson et al., 2010) and also to reduce the risk of cardiovascular disease (Omenn et al., 1996) and stroke (Polidori et al., 2002). However, its use is hampered by its poor solubility in food systems, due to its high hydrophobicity. In addition, lutein is susceptible to rapid degradation that is associated with the reactivity of conjugated double bonds on exposure to light, oxygen and temperature (Mitri, Shegokar, Gohla, Anselmi, & Müller, 2011). The incorporation of lutein into nanocarriers like liposomes may preserve its activity, improve its aqueous dispersibility and enhance its absorption. To date, there have been only a few reports of liposomes loaded with lutein. Tan et al. (2013) obtained lutein-loaded liposomes via the ethanol injection method with an encapsulation efficiency (EE) of 92.0% and a mean diameter of 83.5 nm. Xia, Hu, Jin, Zhao, and Liang (2012) prepared lutein-loaded proliposomes via the supercritical anti-solvent method with a mean diameter of 200 nm and an EE of 90.0%.

The typical preparation of liposomes includes the use of thin film hydration (TFH), ethanol injection, reverse phase evaporation and detergent depletion methods. However, these methods usually result in liposomes with organic solvent/surfactant residues, heterogeneous size distributions and low storage stability. To overcome these drawbacks, in our previous study (Zhao & Temelli, 2015), a single step supercritical carbon dioxide (SC-CO_2) process, which combined the merits of two

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different supercritical methods (Cano-Sarabia et al., 2008; Otake et al., 2006) was developed. Unloaded liposomes were prepared and the effects of pressure, depressurization rate, temperature (Zhao & Temelli, 2015) and liposomal composition (phospholipid and sterol types and concentrations) (Zhao, Temelli, Curtis, & Chen, 2015) on their characteristics were studied. The liposomes obtained were superior to those prepared by the TFH method since they were free of organic solvent and had small particle sizes, an enhanced vesicular intactness and high storage stabilities (Zhao & Temelli, 2015). Our next step was to encapsulate high-value bioactive or nutraceutical ingredients into liposomes and lutein was selected for this purpose with the goal to investigate the lutein-phospholipid interactions and the physiochemical behavior of such multicomponent vesicles. It has been shown that lutein encapsulation into liposomes, especially at high molar concentrations, can affect the physical properties of the bilayer membrane, including the phase transition temperature, degree of order as well as packing parameters, lipid fluidity, polarity and the hydrophobicity of bilayer membranes (Tan et al., 2013; Xia et al., 2015). Thus, the objective of this study was to evaluate the effects of pressure, depressurization rate, temperature and lutein-to-lipid ratio on the properties of the resulting liposomes, including the particle size distribution, zeta potential, encapsulation efficiency, bioactive loading, morphology, phase transition and crystallinity.

2. Materials and methods

2.1. Materials

Soy lecithin obtained from Fisher Scientific (Ottawa, ON, Canada) was used for liposome preparation. Lutein (80%) was purchased from Hangzhou Ningsi Bio-tech (Hangzhou, China). Ethanol (99.8%) and petroleum ether (95%) were obtained from Sigma-Aldrich (Oakville, ON, Canada) and liquid CO_2 (purity of 99.99%) was supplied by Praxair Canada (Mississauga, ON, Canada). Water purified by Milli-Q® ultrapure water purification system (EMD Millipore, Billerica, MA, USA) was used in all experiments.

2.2. Preparation of the phospholipid/lutein suspension

The phospholipid suspension was prepared fresh for each liposome preparation. Soy lecithin (1.33 g, MW: 677.92 g/mol as specified by the supplier) was dispersed in 100 mL Milli-Q water with continuous agitation for 20 min using a magnetic stirrer at 1200 rpm. The final concentration of phospholipids was controlled at 20 mM. Lutein (MW: 568.87 g/mol) was slowly added to obtain different lutein-to-lipid ratios (0.5–20, mol%) and mixed with phospholipids at 1200 rpm in the dark for 1 h. The crude phospholipid/lutein suspension was stored under nitrogen in the dark at 4 °C and used within one week.

2.3. Preparation of liposomes using SC-CO_2 technology

The experimental apparatus (Phase equilibria apparatus, SITEC-Sieber Engineering AG, Maur/Zurich, Switzerland) used for liposome formation was described previously (Zhao & Temelli, 2015). The liposomes were produced in a batch mode. A soy lecithin/lutein suspension (6 mL) was sealed in the high pressure view cell (10 mL) and gently flushed with CO_2. The cell was equilibrated until the set temperature was reached, then pressurized with CO_2 up to the desired pressure. A magnetic stirrer at the bottom was used for thorough mixing of the cell contents at 550 rpm for 1 h. The CO_2-expanded phospholipid/lutein liquid mixture was then depressurized at a constant pressure and rate via a micrometering valve at the bottom of the vessel. To maintain constant pressure inside the vessel, CO_2 was introduced by opening the on/off valve attached to the upper part of the vessel while the liquid phase was being released from the bottom. To achieve a constant depressurization rate, preliminary trials were performed at set depressurization rates and each specific position on the scale of the micrometering valve was recorded. These valve positions were then applied during liposome preparation to achieve the desired depressurization rate. Liposomes were formed when the CO_2-expanded phospholipid/lutein suspension was depressurized from high pressure to ambient conditions and collected from the bottom of the vessel. Different levels of pressure, depressurization rate, temperature and lutein-to-lipid ratio were tested as summarized in Table 1. The factor levels for each parameter were set based on numerous trial runs as well as the findings of our previous studies on unloaded liposomes (Zhao & Temelli, 2015; Zhao et al., 2015). The factor levels, which had the higher impact on each parameter were selected for the presentation of results.

Table 1
Processing conditions for liposome preparation using the SC-CO_2 method.

Factors	Levels	Processing parameters
Pressure (bar)	30, 60, 80, 100, 150, 200, 250, 300	50 °C, 1% lutein and 90 bar/min
Depressurization rate (bar/min)	20, 40, 60, 90, 120, 150, 200	50 °C, 1% lutein and 300 bar
Temperature (°C)	40, 45, 50, 55, 60, 65	1% lutein, 300 bar and 90 bar/min
Lutein-to-lipid ratio (mole %)	0.5, 1, 2, 5, 10, 20	50 °C, 300 bar and 90 bar/min

2.4. Characterization of liposomes

2.4.1. Particle size distribution

The particle size (mean hydrodynamic diameter) and size distribution (polydispersity index, PdI) were determined by Zetasizer Nano ZS instrument (Model ZEN3500, Malvern Instruments, Worcestershire, UK) using the Dynamic Light Scattering (DLS) technique. The viscosity of liposome suspension was reported as 10.2 mPa s at 20 mM phospholipids, 25 °C and shear rate of 3 s^{-1} by Jopski, Pirkl, and Schmidt (1990). Samples were measured at 25 °C using a laser wavelength of 535 nm and scattering angle of 173°.

2.4.2. Zeta potential

The surface charge was measured by the Electrophoretic Light Scattering (ELS) mode of the same instrument used for particle size distribution. Liposome suspension was added slowly into the disposable capillary cell to avoid air bubbles (DTS 1060, Malvern Instruments, Worcestershire, UK). Samples were measured at 25 °C with a laser scattering angle of 13° in the cell. The laser power and electric field were controlled at 60 mW and 25 V/cm, respectively.

2.4.3. Encapsulation efficiency and bioactive loading

The encapsulation efficiency (EE) was determined by quantification of free and encapsulated lutein. Free lutein content (μg) in the aqueous phase was measured according to Tan et al. (2013) with modification. Lutein-loaded liposome samples (0.5 mL) were mixed with petroleum ether (3 mL) and centrifuged at 2000 rpm for 5 min to separate the upper phase. This protocol was repeated and the two supernatants were combined. The absorbance of total supernatant was measured in a semi-micro quartz cuvette (Hellma 6040-UV, Hellma Analytics, Müllheim, Germany) using a UV–Visible spectrophotometer (Jenway 6305, Bibby Scientific Ltd., Staffordshire, UK). Prior to the absorbance measurement, a full spectrum scan of lutein in petroleum ether was performed and the peak wavelength was determined as 442 nm. A calibration curve of absorbance versus lutein concentration was established to determine the concentration of free lutein (μg/mL). The free lutein content (μg) was calculated via the free lutein concentration (μg/mL) in petroleum ether times the volume (mL) of the supernatant obtained.

The encapsulated lutein content (μg) in the liposomal phase was