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Nano-encapsulation of coenzyme Q_{10} using octenyl succinic anhydride modified starch ☆

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ABSTRACT

Octenyl succinic anhydride modified starch (OSA-ST) was used to encapsulate coenzyme Q_{10} (CoQ_{10}). CoQ_{10} was dissolved in rice bran oil and incorporated into an aqueous OSA-ST solution. High pressure homogenisation of the mixture was conducted at 170 MPa for 56 cycles. The resulting emulsion had a particle size range of 200–300 nm and the absolute zeta potential varied between 8.4 and 10.6 mV. CoQ_{10} retention of the emulsion and freeze dried products, determined by a hexane rinse, was 98.2%. Reconstitution of the freeze dried product in McIlvaine citrate–phosphate buffers with pH values of 3–5 and temperatures at 4 and 25 °C had very little effect on the range and distribution of the nanoparticles' size. The inflection point of the zeta potential and pH plot occurred at the first p*K*a of succinic acid (pH 4.2), indicating succinate as the main influence over zeta potential.

1. Introduction

Nano-encapsulation or emulsification of bioactive molecules has been shown to be an effective way of increasing the bioavailability of drugs and nutrients (Kreuter, 2007; Singh & Lilliard, 2009). The encapsulation strategy, unlike other methods to increase bioavailability, e.g. changing molecular structure (Ghose, Viswanadhan, & Wendoloski, 1999; Lipinski, Lombardo, Dominy, & Feeney, 2001) or intravenous delivery, is more nonspecific, and has been proven to be effective on the microscale (Bayer's Pro-Release™). It should prove more effective on the nanoscale (De Jong & Borm, 2008) because of the increase in the surface area to mass ratio and rapid uptake. The chemical makeup of the carrier is as important as the molecule delivered, since the chemical and physical properties of the nanoparticles and the bulk material can be very different, even though the molecular composition is identical. Mechanical processing, such as ultrasound (Jafari, He, & Bhandari, 2006, 2007), high pressure homogenisation (Liu, Wu, Chen, & Chang, 2009), and microfluidization (Jafari et al., 2006, 2007), are common techniques in the formation of nanoemulsions and nanoparticles.

OSA modified starch, patented by Caldwell and Wurzburg (1953) and Billmers and Mackewicz (1997) is a low-cost encapsulating/emulsifying agent, which has been granted GRAS status for OSA modifications up to 3% of the starch weight. The octenyl succinate group in conjunction with starch's polysaccharide backbone confers the ability to form stable oil-in-water (o/w) emulsions. The use of modified starch, with its α (1–4) glucosyl backbone, is ideal for gut absorption of any encapsulated material through the small intestine. The introduction of the octenyl side chain significantly reduces the activity of α-amylase (Viswanathan, 1999), tempering the release of the material due to starch hydrolysis after consumption.

Coenzyme Q_{10}, currently the third most consumed nutritional supplement after fish oil and multivitamins, has been chosen as the molecule of interest for this study. This endogenous biomolecule is an integral part of the mitochondrial membrane's electron transport chain and is therefore essential for aerobic metabolism.

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The poor bioavailability, as an essential nutrient, and its colour (for easy quality and quantity control purposes) has made CoQ_{10} an attractive target for researchers designing a comprehensive system for the encapsulation strategy. No LD_{50} (lethal dose to kill 50% of test animals) is given for CoQ_{10}, as the minor side effects, such as indigestion, of megadoses of CoQ_{10} are more associated with the copious amounts of oil used to solvate the CoQ_{10} (Hathcock & Shao, 2006). Despite the human body's ability to synthesise CoQ_{10}, internal (genetics) or external (statins) factors can lower physiological concentrations of CoQ_{10}. The lack of CoQ_{10} is symptomatic in cells and tissue normally rich in mitochondria, such as muscle and nerve tissue, resulting in various types of myopathy and neuropathy, and CoQ_{10} supplements have shown to improve these patients' symptoms (Boitier et al., 1998; Di Giovanni et al., 2001; Ogasahara, Engel, Frens, & Mack, 1989; Rötig et al., 2000; Sobreira et al., 1997; Thompson, Clarkson, & Karas, 2003). CoQ_{10} deficiencies have also been cited in Parkinson's, periodontal disease, type 2 diabetes and hypertension, where oxidative stress may play a significant role (Molyneux, Young, Florkowski, Lever, & George, 2008). CoQ_{10} antioxidative properties can also be used as a treatment to reduce oxidative radicals brought on by ionising radiation or radiation poisoning. These studies involved massive doses, upwards of several grams of CoQ_{10} in an oil suspension to saturate the plasma, because the lipophilic nature of CoQ_{10} results in poor gut absorption and greater than 90% passes through the digestive tract unabsorbed. The absorption of CoQ_{10} or other lipophilic nutrients improves by ingesting supplements with the intake of food and the formation of an initial emulsion (Ochiai et al., 2007).

Most diets do not include a large variety or quantity of animal meat and organs (Pravst, Žmitek, & Žmitek, 2010), and so CoQ_{10} supplements are required to observe any beneficial clinical effects. While there are many types of supplements that offset CoQ_{10} hydrophobicity and bioavailability, only β and γ cyclodextrins have demonstrated increasing CoQ_{10} levels *in vivo* (Žmitek et al., 2008). Improvements in bioavailability and dissolution will hopefully mitigate the need for the large doses currently used to obtain ideal plasma and intracellular levels of CoQ_{10}, without compromising taste or texture of target beverages.

The main objective of this research is to use OSA modified starch to encapsulate or entrap CoQ_{10} and characterise the product. Stability at different pH values were chosen to mimic different fruit juices, tea and coffee. Effects of high pressure homogenisation (HGH), pH and temperature on particle size and absolute zeta potential (ζ_{abs}) were analysed over 3 weeks.

2. Materials and methods

2.1. Materials and equipment

Octenyl succinylated starch (OSA-ST), Hi-Cap 100 (National Starch, Bridgewater, NJ), coenzyme Q_{10} (Sigma–Aldrich, St. Louis, MO), hexanes (Baker, Phillipsburg, NJ) and rice bran oil (Loriva, Dayville, CT) were used without modification or purification. The freeze dryer used in this study was a Virtis Genesis 25ES (Gardiner, NY). All water used in this study was deionised ($\geqslant$18 MΩ with a ZyzaTech Lab Five (Seattle, WA) filtration system that had activated carbon (#10,817) and deionizing resin (#10,816) cartridges from Benchmark Technologies placed in series, in a pressurised system (20–30 psi). The CO_2 supercritical fluid extraction system used was from ISCO (Lincoln, NE) and consisted of an extractor (#220), controller (#200), syringe pump (#260D), coaxial temperature controlled restrictors (#68-3937-006) and 10 ml extraction cartridges (#60-3868-046).

2.2. Initial emulsion

Batches of the octenyl succinylated starch CoQ_{10} emulsion (OSOQ10) product were made by dissolving 20 g of modified starch in 80 ml of deionised water overnight in a 250 ml beaker. CoQ_{10} (450 mg) was dissolved in 4.0 g of rice bran oil by sonication in an ice bath and then dispersed in the OSA-ST solution by syringe (5 ml, 1.5 inch, 18 gauge) injection over 2 min at 8 k rpm (Ultra-Turrax T25, IKA, Staufen, Germany). After allowing the oil phase to disperse, the mixture underwent further mixing at 25 k rpm for 5 min. The foam dissolved slowly while sonicating and intermittent stirring in an ice bath for 30 min.

2.3. High pressure homogenisation

The OSOQ10 emulsion was cycled five to six times through the high pressure homogeniser at 170 kPa (Emulsiflex-C5, Avestin Inc., Ottawa, Canada). The output flowed through 1/8″ ID Masterflex 96,400 tubing. During the optimisation process, samples were taken out *pro rata* based on a flow rate of 40 ml/min. A stainless steel coil interjected in the tubing was placed in an ice bath during processing. The samples were flash-frozen in liquid N_2, freeze dried and ground into a fine powder, which readily dispersed in aqueous solutions.

2.4. Freeze drying

Kaasgaard and Keller (2010) noted changes in the size distribution profile between the homogenised solution and the reconstituted freeze dried product. They speculated that the formation of ice crystals during freezing might cause the particles in the nano-emulsion to coalesce. To avoid the potential problems of ice crystal formation in the emulsion, liquid nitrogen was used to 'flash freeze' the emulsion to a vitrified state. The parameters of the freeze dryer were set at less than 130 mTorr at −25 °C (shelf) for the first 24 h and 0–25 °C for the next 24 h with the condenser at a −55 °C.

2.5. Scanning electron microscopy (SEM)

The morphology of the OSOQ10 particles was visualised by an environmental scanning electron microscope (FEI XL-30 ESEM, FEI-Phillips, Hillsboro, OR). The freeze dried OSOQ10 samples were mounted on standard 0.5 inch Cambridge SEM stub using double-sided adhesive tabs. The stub was gently dipped into the sample and tapped to remove loose particles. The specimen was coated with gold/palladium (60%/40%) using a Hummer II Sputter Coater, (Technics Inc, Alexandria, VA) before imaging at 10–12 kV with the XL30 ESEM.

2.6. Particle size and stability analysis

Particle sizes and ζ potentials were measured using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK). Samples were taken from the homogeniser (100 μl), diluted with water (2.9 ml), stirred and allowed to equilibrate for 20–30 min at 25 °C. Initial analysis was done in deionised water, however buffers were utilised for the stability analysis. The pH range and the buffer type were chosen to reflect the physiochemical profile of coffee, tea and fruit beverages. Freeze dried samples were reconstituted in two by five sets to a concentration of 10 mg/ml in deionised water and 5 mg/ml in 0.1 M citrate–phosphate buffers at pH 3, 5 and 7 (McIlvaine, 1921). The pH range and the buffer type were chosen to reflect the physiochemical profile of fruit beverages. One set was stored at 4 °C and another set was stored at 25 °C. A sample of each set was analysed every 4 days for about 3 weeks and then