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Formation and characterization of filled hydrogel beads based on calcium alginate: Factors influencing nanoemulsion retention and release

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ABSTRACT

Filled hydrogel particles fabricated from natural lipids and biopolymers can be utilized as tailor-made encapsulation and delivery systems. In the present study, the impact of nanoemulsion fabrication method (low energy *versus* high energy) on the retention and release of a lipophilic bioactive component (curcumin) and of lipid droplets from calcium alginate beads was investigated. Initially, curcumin was dissolved within an oil phase, and then the oil phase was encapsulated within the hydrogel beads using a two-step process. First, nanoemulsions composed of the same constituents (medium chain triglycerides, Tween 60, curcumin, and phosphate buffer) were prepared using either low energy (spontaneous emulsification) or high energy (microfluidization) homogenization. Second, the nanoemulsions were mixed with alginate solutions (0.25–1.5%), and then the resulting mixture was dripped into calcium solutions (10–500 mM) to form filled hydrogel beads. Unloaded beads became more spherical and rigid with increasing alginate and calcium concentrations. Turbidity and spectrophotometry measurements showed that the amount and extent of curcumin and lipid droplet release from the hydrogel beads decreased with increasing alginate and calcium concentration, which was attributed to a reduction in the pore size of the hydrogel matrix. In particular, a high amounts of curcumin released were observed in delivery systems containing high Tween 60 concentrations, which was attributed to rapid diffusion of curcumin-loaded surfactant micelles out of the beads. These results have important implications for the design of delivery systems to entrap and control the release of lipophilic bioactive components within filled hydrogel particles.

1. Introduction

In recent years, tailor-made delivery systems have been investigated for the encapsulation, protection, and controlled release of bioactives and nutrients in the food, cosmetic, and pharmaceutical industries (McClements, Decker, Park, & Weiss, 2009; Paques, van der Linden, van Rijn, & Sagis, 2013, 2014; Zeeb, Saberi, Weiss, & McClements, 2015). There has been considerable interest in the utilization of hydrogel beads because delivery systems with a wide range of functional attributes can be fabricated from food-grade hydrocolloids, such as gelatin, starch, carrageenan, pectin, or alginate (Fundueanu, Nastruzzi, Carpov, Desbrieres, & Rinaudo, 1999; Hills et al., 2000). In particular, sodium alginate (derived from brown algae) has a number of attributes that make it particularly suitable for forming hydrogel beads: (i) simple preparation method; (ii) compatibility with other ingredients; (iii) non-toxicity, high availability and low cost; and, (iv) biodegradability (Draget, Smidsrød, & Skjåk-Bræk, 2005; Shilpa, Agrawal, & Ray, 2003).

Alginate is an unbranched biopolymer consisting of (1 → 4) linked β-D mannuronic acid (M) and α-L-guluronic acid (G) residues varying greatly in composition and sequence (Draget et al., 2005; Velings & Mestdagh, 1995). It can form ionotropic gels in the

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presence of divalent or trivalent cations such as Ca^{2+}, St^{2+}, Ba^{2+}, or Fe^{3+} due to the formation of cationic bridges between the guluronic rich entities along the biopolymer backbone leading to the characteristic egg-box structure (Li, Hu, Du, Xiao, & McClements, 2011; Shilpa et al., 2003). In general, the bioactive to be encapsulated is mixed with an alginate solution, and then the mixture is injected into a solution containing divalent ions, which results in the formation of hydrogel beads (Fundueanu et al., 1999).

Hydrogel beads composed of alginate allow the entrapment of a wide range of environmentally sensitive bioactives since the interior is known to be chemically inert (Griffith, 2000). In addition, alginate beads can enhance the physical and biochemical stability of encapsulated lipid droplets by modulating the hydrogel matrix porosity and degradability (Gombotz & Wee, 1998; Paques, van der Linden, van Rijn, & Sagis, 2014). The encapsulation efficiency of lipid droplets has previously been shown to depend on the degree of alginate crosslinking (Chan, 2011). On the other hand, the incorporation of lipid droplets has been shown to increase alginate bead size (Chan, Lim, & Heng, 2000). The functionality of filled alginate beads can be further tailored by coating them with other biopolymers, such as chitosan (Peniche, Howland, Carrillo, Zaldıvar, & Argüelles-Monal, 2004).

Recent research has focused on the utilization of hydrogel beads to improve the oral bioavailability of lipophilic compounds, as well as to control the release of water-insoluble molecules within the human gastrointestinal tract. For example, it has been shown that the release of free fatty acids during lipid digestion under simulated gastrointestinal conditions was reduced from 100% to 12% when the lipid droplets were encapsulated within calcium alginate beads (Li et al., 2011; Li, Kim, Park, & McClements, 2012). The authors proposed that the bead matrix was able to restrict the access of digestive enzymes and other surface-active components to the surface of the encapsulated lipid droplets, which resulted in a decreased rate and extent of lipid digestion. In general, various studies have demonstrated that the encapsulation efficiency depends on bead properties such as size, shape, swelling behaviour, and surface morphology (Lee, Ravindra, & Chan, 2013).

The objective of the present study was to establish a better understanding of the factors influencing the retention and release of a model lipophilic bioactive (curcumin) and nanoemulsion droplets from filled hydrogel beads. Curcumin was used because it has a yellowish colour that can conveniently be used to monitor its release from hydrogel beads. In addition, curcumin is an important bioactive component that may need to be incorporated into functional foods in the form of emulsions or nanoemulsions (Ahmed, Li, McClements, & Xiao, 2012). Nanoemulsions were prepared using two emulsification techniques based on different physicochemical principles: (i) spontaneous emulsification was used to produce nanoemulsions using a low energy method; (ii) microfluidization was used to produce nanoemulsions using a high energy method. The nanoemulsions were then mixed with alginate solutions that were dripped into calcium solutions to induce hydrogel bead formation. Our hypothesis was that the retention and release of the nanoemulsions would depend on their fabrication method, as well as the properties of the beads (such as alginate and calcium concentration). The information obtained from this study will be useful for designing hydrogel beads that can release encapsulated bioactive lipids in response to different environmental conditions.

2. Materials and methods

2.1. Materials

Sodium alginate (alginic acid sodium salt from *Macrocystis pyrifera*, #50K0180, medium viscosity, 20–40 cps of 1% aqueous solution) was purchased from Sigma–Aldrich Co. (St. Louis, USA). The alginate used in this study was composed of 61% β-D-mannuronic acid (M) and 39% α-L-guluronic (G), which is equal to an M/G ratio of 1.56. Curcumin (purity > 98%) was obtained from Acros Organics (New Jersey, USA) and used without further purification. Medium chain triglyceride (MCT) oil (MIGLYOL®812) was purchased from Warner Graham Company (Sasol GmbH, Germany). Polysorbate 60 (Tween 60), calcium chloride anhydrous (purity > 96.0%), and dimethylsulfoxide (DMSO) were obtained from Sigma–Aldrich Co. (St. Louis, USA). Double-distilled water was used for the preparation of all samples. All concentrations are expressed as mass percentage (% w/w).

2.2. Solution preparation

An emulsifier solution was prepared by dispersing semi-solid Tween 60 into buffer solution (5 mM phosphate, pH 7) followed by heating to 40 °C to ensure complete dissolution. A stock alginate solution (3%) was made by dispersing alginate powder into double-distilled water and stirring overnight. Stock hardening solution was prepared by dissolving 1 M $CaCl_2$ into double-distilled water followed by stirring for at least 30 min.

2.3. Emulsion preparation

2.3.1. Low-energy nanoemulsions (LEN)

Nanoemulsion formation was carried out using the spontaneous emulsification procedure described in a previous study (Saberi, Fang, & McClements, 2013). Briefly, spontaneous emulsification was performed by addition of an organic phase to an aqueous phase with constant magnetic stirring (600 rpm) at 25 °C. The organic phase consisted of 10% MCT (containing 0.067% curcumin) and 9% Tween 60, while the aqueous phase (81%) consisted of 5 mM phosphate buffer (pH 7.0). The oil (10 g MCT) and Tween 60 (9 g) were first mixed together to form an organic phase, which was then slowly poured into 81 g of aqueous phase over a 15 min period with continuous stirring.

2.3.2. High-energy nanoemulsions (HEN)

Emulsions were prepared by microfluidization, which involved homogenizing 10% MCT (containing 0.067% curcumin) and 90% aqueous phase (1% Tween 60, 5 mM phosphate buffer, pH 7) using a high shear mixer (Barmix, Biospec Products, Bartlesville, OK) for 3 min followed by five passes at 10,000 psi (68.95 MPa) through a microfluidizer (M-110L, Microfluidcs, Newton, MA). After microfluidization, one part of the HE-nanoemulsion was mixed with aqueous Tween 60 solution to obtain a system with the same final surfactant concentration (9%) as the LE-nanoemulsion referred to as "*HEN + T60*".

2.4. Emulsion characterization

Dynamic light scattering was used to determine the particle diameters of nanoemulsions produced by spontaneous emulsification and microfluidization (Nano ZS, Malvern Instruments, Malvern, UK). Samples were diluted to a droplet concentration of approximately 0.005% with 5 mM phosphate buffer (pH 7) to prevent multiple scattering effects. The foundation of this technique is based on the scattering of light by moving particles due to Brownian motion in a liquid (Dalgleish & Hallett, 1995). The movement of the particles is then related to the size of the particles. The instrument reports the mean particle diameter (z-average) and the polydispersity index (PDI) ranging from 0 (monodisperse) to 0.50 (very broad distribution).