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Food-grade filled hydrogels for oral delivery of lipophilic active ingredients: Temperature-triggered release microgels

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ABSTRACT

Delivery systems are often needed to encapsulate lipophilic active agents, protect them during storage, and then release them within the mouth. In this study, gelatin and caseinate were used to fabricate temperature-sensitive filled hydrogel particles. Filled hydrogel microspheres were formed by electrostatic complexation of caseinate and gelatin in the presence of caseinate-coated lipid droplets. This was achieved by mixing aqueous 1% sodium caseinate and 1% gelatin solutions (volume ratio 1:2) at pH 5.8 with an oil-in-water emulsion. The majority of lipid droplets were trapped within the hydrogel microspheres. Turbidity and viscosity measurements of the hydrogels indicated that hydrogel particles dissociated upon heating because of gelatin melting (around 35 °C). Light scattering and confocal fluorescence microscopy indicated that lipid droplets were released from the gelatin-based hydrogel particles after oral processing, which was attributed to hydrogel melting under simulated mouth conditions. Our results suggest that hydrogel particles based on electrostatic complexation of sodium caseinate and gelatin could be useful as oral delivery systems for lipophilic active agents.

1. Introduction

There is increasing interest within the food and biomedical industries to incorporate lipophilic bioactive agents into functional food and medical food products, e.g., vitamins, nutraceuticals, pharmaceuticals, antimicrobials, flavors, and colors (Augustin & Hemar, 2009; McClements, 2013; McClements & Xiao, 2014; Velikov & Pelan, 2008). However, the poor water-solubility and chemical stability of many lipophilic bioactive agents make them difficult to incorporate into food matrices (Augustin & Sanguansri, 2012; Williams et al., 2013, Yao et al., 2014). The low solubility of these bioactive agents in aqueous environments means that they tend to phase separate (Li et al., 2012), whereas their chemical instability means that they may degrade within the product prior to consumption, thereby reducing their efficacy (Qian et al., 2012). It is therefore necessary to design food-grade delivery systems to improve the dispersibility and chemical stability of lipophilic bioactive agents in food matrices (Augustin & Hemar, 2009; McClements, 2014; Velikov & Pelan, 2008).

Flavor perception (taste and aroma) throughout the eating process is a key factor in determining the desirable sensory attributes of many food products (Madene et al., 2006; Pothakamury & BarbosaCanovas, 1995; Sorensen et al., 2003). It is often important to control the release profile of lipophilic flavors within the mouth to provide the desired overall sensory experience (Doyennette et al., 2014). Other types of lipophilic bioactive agents may also need to be released within the mouth after consumption of medical or functional foods, e.g., some oil-soluble drugs (Porter et al., 2008; Pouton, 2006), and antimicrobials for oral health (Huang et al., 2010; Loesche, 1999). It is therefore important to food-grade effective delivery systems that can: encapsulate efficacious levels of lipophilic bioactive agents; easily be incorporated into food or beverage matrices; protect the bioactive agents from chemical degradation during storage; and release them within the mouth after ingestion (often at a controlled rate) (Augustin & Sanguansri, 2012; McClements, 2014).

For many applications, delivery systems are needed that can be incorporated into high-moisture food matrices, such as those in beverages, yogurts, dressings, sauces, creams, soups, dips, and desserts. A variety of different emulsion-based delivery systems have been developed to encapsulate lipophilic bioactive agents, including emulsions, nanoemulsions, multiple emulsions, multilayer emulsions, filled hydrogel particles, and solid lipid nanoparticles (Gershkovich et al., 2008; Han et al., 2009; Lee et al., 2008; McClements, 2010; McClements et al., 2008; Nordly et al., 2009; Semalty et al., 2009). Each of these delivery systems has their own advantages and disadvantages in terms of cost, ease of preparation, in-product stability, product compatibility, ingredient utilization, and functional performance (McClements, 2014).

In this study, we focus on the utilization of filled hydrogel particles, which consist of lipid droplets trapped within hydrogel microspheres. Initially, the lipophilic bioactive agents are dissolved in an oil phase,

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and then an oil-in-water emulsion or nanoemulsion is formed using an appropriate homogenization method (Yao, Xiao, & McClements, 2014). In principle, hydrogel microspheres can be formed using various different fabrication methods depending on the final characteristics of the particles required, including extrusion, templating, molding, thermodynamic incompatibility, and complexation (Matalanis et al., 2011; Shewan & Stokes, 2013). Electrostatic complexation is a relatively straightforward method that can easily be implemented commercially, since it simply relies on the formation of complexes between polymers in solution that have opposite charges (Kayitmazer et al., 2013; Kizilay et al., 2011). Filled hydrogel particles can be designed to control the protection and release of lipophilic bioactive agents by controlling the composition and structure of the hydrogel matrices. The properties of hydrogel particles formed using electrostatic complexation can be modulated by selecting biopolymers with different molecular characteristics (such as electrical properties, molecular weight, and structure) and by modulating preparation conditions (such as pH, salt, and stirring).

The design of delivery systems that can control the release of encapsulated bioactive agents within the mouth depends on an understanding of oral processing (Stieger & van de Velde, 2013; Van Aken et al., 2011; van Vliet et al., 2009). Numerous factors can potentially influence the behavior of delivery systems within the oral cavity, including: in-mouth warming or cooling; saliva composition, volume, and flow rate; mixing, disruption, and frictional forces; and pH changes. In a recent study, we fabricated hydrogel particles by trapping lipid droplets within electrostatic complexes formed by sodium caseinate and alginate at pH 5 (Zhang et al., 2015). We showed that these lipid droplets could be retained within hydrogel particles during storage, but released from the hydrogel matrix under conditions simulating oral processing. In that system, the main driving force for hydrogel disruption and droplet release was the pH change in the mouth. Under storage conditions (pH 5), the electrostatic complexes were stable due to electrostatic attraction between anionic pectin and cationic patches on the caseinate molecules. However, under oral conditions (pH 7), the complexes dissociated due to electrostatic repulsion between the anionic pectin and anionic caseinate molecules. These results suggested that filled hydrogel particles based on electrostatic complexation of two oppositely charged biopolymers might be useful for oral delivery of lipophilic bioactive agents.

The aim of the current study was to develop filled hydrogel particles that could retain encapsulated lipid droplets within a product, but release them under simulated oral conditions due to a temperature trigger. We prepared hydrogel particles with these characteristics by incorporating lipid droplets into electrostatic complexes formed using two food-grade proteins: gelatin and casein. The hydrogel particles formed in this study can be considered to be a kind of microsphere or microgel. A potential advantage of using caseinate to form the hydrogel particles is that it can stabilize encapsulated polyunsaturated lipids against oxidation, which has been attributed to its antioxidant capacity (Matalanis et al., 2012; Zhang et al., 2014). On the other hand, a potential advantage of using gelatin for fabricating oral delivery systems, is that it can form gels that melt in the mouth, i.e., undergo a gel-to-sol transition around body temperature (Malone & Appelqvist, 2003). The main novelty of this study is the fabrication and testing of a food-grade delivery system that can retain lipophilic components within a product, but then release them within the mouth after ingestion. The information obtained from this study may be useful for the rational design of more effective delivery systems for the encapsulation, protection, and release of lipophilic bioactive agents.

2. Materials and methods

2.1. Materials

Corn oil was purchased from a local supermarket and used without further purification. Sodium caseinate powder was obtained from the American Casein Company (MP Biomedicals LLC). As stated by the manufacturer, this ingredient had a protein content of 91.4% and moisture content of 5.0%. Type A gelatin (100 bloom, extracted from pork skin) was kindly donated by GELITA American Company (Sergeant Bluff, IA). Double distilled water was used to prepare all solutions.

2.2. Methods

2.2.1. Emulsion preparation

An oil-in-water (O/W) emulsion stabilized by sodium caseinate (NaC) was prepared as a stock emulsion. Initially, an aqueous emulsifier solution was prepared by dispersing 1% (*w*/*w*) NaC powder into 10 mM phosphate buffer solution (pH 7) with continuous stirring at 700 rpm for 2 h (40 °C). A coarse emulsion was then prepared by blending corn oil (10% *w*/*w*) and emulsifier solution (90% *w*/*w*) together using a high-shear mixer for 2 min (Model 985370-395, Tissue Tearor, Biospec Products Inc., Bartlesville, OK). The size of the droplets in the resulting coarse emulsion was further reduced by passing it three times through a high-pressure homogenizer (Microfluidics Microfluidizer M-110P, Newton, MA USA) at 12,000 psi. The emulsion produced was then diluted so that it had a corn oil content of 6% (*w*/*w*) for the further experiments.

2.2.2. ζ-potential measurements

The electrical characteristics (ζ-potential) of solutions of caseinate, gelatin, and their mixture were measured at different pH values (pH 5 to 10) using a particle electrophoresis instrument (Zetasizer Nano ZA series, Malvern Instruments Ltd. Worcestershire, UK). Samples were diluted using 10 mM phosphate buffer (at the same pH as the sample) prior to analysis so that the instrument attenuation value was within the optimum range (5 to 10). All measurements were made on at least two freshly prepared samples and each sample was measured in duplicate.

2.2.3. Turbidity measurements

The turbidities of dispersions containing caseinate and gelatin were determined using a UV–visible spectrophotometer at 600 nm (Ultrospec 3000 pro, Biochrom Ltd., Cambridge, UK). The samples were contained within 1 cm path length optical cells, and phosphate buffer was used as a control. Turbidity measurements were carried out on at least two freshly prepared samples. The turbidity of biopolymer solutions (pH 5.8) with increasing temperature was analyzed using an UV/visible spectrophotometer at 600 nm (Ultraspec 3000 pro, Biochrom Ltd., Cambridge, UK). Temperature scanning was carried out from 20 to 60 °C at a heating rate of 1 °C per minute.

2.2.4. Unfilled hydrogel microsphere preparation

Initially, 1% (*w*/*w*) NaC and 1% (*w*/*w*) gelatin solutions were prepared by dissolving weighed amounts of biopolymers into phosphate buffer (pH 7). Gelatin solutions were stored in a refrigerator overnight and heated to melt them prior to use. 2 M sodium hydroxide was used to adjust the dissolved biopolymer solution to pH 10. Then these two stock solutions and phosphate buffer were mixed together at different volume ratios with continuous stirring to form final compositions of 0.33% sodium caseinate and 0.67% gelatin (mass ratio 1:2). The mixed biopolymer solutions were then adjusted back to pH 10.0 (if necessary) using 0.1 M sodium hydroxide. The mixtures were then acidified to pH 5.8 using 1 M citric acid at a rate of 1 drop/10 s with continuous stirring at 500 rpm.

2.2.5. Filled hydrogel microsphere preparation

Stock solutions of 2% sodium caseinate and 1% gelatin were prepared separately in 10 mM phosphate buffer at pH 7 and stirred until fully dissolved. 2 M sodium hydroxide was used to adjust the dissolved biopolymer solutions to pH 10. After pH adjustment, 6% (*w*/*w*) caseinate-stabilized oil-in-water emulsion (prepared in the Section 2.2.1) and 2% caseinate solution were mixed together (1:1 volume ratio) with