Encapsulation of curcumin in polysaccharide-based hydrogel beads: Impact of bead type on lipid digestion and curcumin bioaccessibility

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Abstract

Curcumin was incorporated into three different delivery systems: free lipid droplets; lipid-loaded alginate beads; and, lipid-loaded carrageenan beads. Hydrogel beads were fabricated from polysaccharides (alginate or carrageenan) using an injection method combined with ion gelation (calcium or potassium, respectively). The delivery systems were passed through a simulated gastrointestinal tract (GIT) that included mouth, stomach, and small intestine phases. Light scattering and microscopy indicated that carrageenan beads had a relatively fragile structure that was easily disrupted in the GIT and released the encapsulated lipid droplets and curcumin. Conversely, alginate beads had a robust structure that remained relatively intact throughout the GIT and retained the lipid droplets and curcumin. The rate and extent of lipid digestion decreased in the following order: free lipid droplets > carrageenan beads > alginate beads. Curcumin bioaccessibility followed a similar order: free lipid droplets (73%) > carrageenan beads (33%) > alginate beads (16%). These results suggest that lipid droplets must be digested by lipase in order to release the encapsulated curcumin and to form mixed micelles capable of solubilizing the released curcumin. Overall, our results show that delivery systems with different structures and compositions can be designed to control the release of lipids and hydrophobic nutraceuticals in the GIT, which may be advantageous for the development of certain functional food products.

Keywords: carrageenan; alginate; hydrogel beads; curcumin; digestion; bioaccessibility

1. Introduction

Turmeric is a commonly used pigment, spice, and nutraceutical in foods due to its intense yellowish color, unique flavor profile, and biological activities (Prasad, Gupta, Tyagi, & Aggarwal, 2014; Syed, Liew, Loh, & Peh, 2015). Curcumin is the major bioactive agent in turmeric, and it consists of three closely related lipophilic molecules that have a number of phenolic groups and conjugated double bonds (Heger, van Golen, Broekgaarden, & Michel, 2014; Prasad, et al., 2014). The potential pharmaceutical activity of curcumin has been established for numerous diseases, including multiple myeloma, rheumatoid arthritis, cystic fibrosis, inflammatory bowel disease, and colon cancer (Heger, et al., 2014; Prasad, et al., 2014; Wilken, Veena, Wang, & Srivatsan, 2011). Previous research suggests that the health benefits of curcumin are associated with a number of biological activities, including anti-oxidative, anti-inflammatory, anti-microbial, anti-parasitic, anti-mutagenic, and anti-tumor activities (Ringman, Frautschy, Cole, Masterman, & Cummings, 2005; Sharma, Gescher, & Steward, 2005; Wilken, et al., 2011). Another benefit of curcumin is that it has a low toxicity even when ingested at relatively high doses. Consumer demand for health-promoting foods containing fewer synthetic additives has made curcumin of particular interest as a natural nutraceutical ingredient (Anand, Kunnumakkara, Newman, & Aggarwal, 2007).

However, there are a number of physicochemical characteristics of curcumin that currently limit its incorporation into many functional food products, such as its intense color, strong flavor, low water-solubility, and chemical instability (Letchford, Liggins, & Burt, 2008). Moreover, curcumin is rapidly metabolized within the gastrointestinal tract (GIT), which limits its potential beneficial biological effects (Anand, et al., 2007; Sharma, Steward, & Gescher, 2007). Some of these limitations can be overcome by appropriate food product selection, *e.g.*, appearance and flavor problems can be overcome by incorporating curcumin into highly colored and spicy products. Other limitations can be overcome by encapsulating curcumin within food-grade colloidal delivery systems or by mixing it with colloidal excipient systems (Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007; Margulis, Magdassi, Lee, & Macosko, 2014; Sahu, Kasaju, & Bora, 2008; Tapal & Tikku, 2012; Tiyafoonchai, Tungpradit, & Plianbangchang, 2007; Yu, Shi, Liu, & Huang, 2012; Zou, Liu, Liu, Xiao, & McClements, 2015a). A colloidal delivery

84 system consists of small particles (typically comprised of lipids, phospholipids,
85 surfactants, and/or biopolymers) that contain the curcumin(McClements, 2015b). On
86 the other hand, a colloidal excipient system consists of small particles that are mixed with
87 another food containing curcumin(Zou, et al., 2015). These small particles are
88 specifically designed to boost the bioavailability of curcumin. Colloidal delivery
89 systems can be designed to facilitate the incorporation of curcumin into commercial food
90 products, while both colloidal delivery and excipient systems can be designed to improve
91 curcumin's chemical/biochemical stability and to control its fate within the GIT.

92 Previous studies have demonstrated the potential of improving the bioavailability of
93 curcumin using colloidal delivery systems (Ahmed, Li, McClements, & Xiao, 2012;
94 Anand, et al., 2010; Patel & Velikov, 2011; Sun, et al., 2012; Ting, Jiang, Ho, & Huang,
95 2014; Yu & Huang, 2012) or colloidal excipient systems (Zou, Liu, Liu, Xiao, &
96 McClements, 2015; Zou, et al., 2015a; Zou, et al., 2015). Emulsion-based systems are
97 particularly useful for this purpose because their composition and structures can be
98 designed to alter the bioaccessibility, absorption, and transformation of lipophilic
99 bioactives (McClements & Xiao, 2014). In particular, the lipid phase in emulsions can be
100 designed to rapidly digest within the small intestine and form mixed micelles capable of
101 solubilizing and transporting lipophilic bioactives(Porter, Pouton, Cuine, & Charman,
102 2008; Yao, Xiao, & McClements, 2014). In addition, emulsions are already integral parts
103 of many commercial food and beverage products(McClements, 2015a), and so
104 emulsion-based delivery or excipient systems can easily be incorporated into a wide range
105 of foods and beverages. One of the main limitations of conventional oil-in-water emulsions
106 for this purpose is that they only have limited scope for controlling the stability and release
107 of any encapsulated bioactive agents because the lipid droplets are only coated by a thin
108 layer of emulsifier molecules. This limitation can be overcome by trapping the lipid
109 droplets inside hydrogel beads ("microgels").

110 Hydrogel beads suitable for utilization in the food industry are usually fabricated from
111 food-grade biopolymers, such as proteins and/or polysaccharides (Chen, Remondetto, &
112 Subirade, 2006; Joye & McClements, 2014; Shewan & Stokes, 2013). These beads can be
113 fabricated using numerous approaches, including injection, coacervation, thermodynamic

incompatibility, antisolvent precipitation, templating, and molding methods(Matalanis, Jones, & McClements, 2011). The injection-gelationmethod is one of the simplest and most widely used approaches for the encapsulation, protection, and delivery of food-grade bioactive components such as nutrients, nutraceuticals, and vitamins. In this method, a biopolymer solution containing the bioactive component is injected into another “hardening” solution under conditions that promote the gelation of the injected biopolymer. This procedure results in the formation of a hydrogel bead with the bioactive components trapped inside. The nature of the hydrogel matrix surrounding the bioactive can be designed to improve its physical and chemical stability, as well as to control its GIT fate.

In the present study, curcumin was initially solubilized within the lipid phase of an oil-in-water nanoemulsion. The curcumin-loaded lipid droplets were then used as delivery systems themselves, or they were incorporated into hydrogel beads fabricated from either carrageenan or alginate. The filled hydrogel beads were formed by mixing the lipid droplets with a polysaccharide solution (alginate or κ -carrageenan), and then injecting the resulting mixture into a solution of positively charged ions (Ca^{2+} or K^+ , respectively). The cations act as salt bridges to promote gelation of the anionic polysaccharides molecules. Carrageenan and alginate were used to form the hydrogel beads because they are already widely utilized as food-grade ingredients. Furthermore, these two polysaccharides were used because they are expected to form hydrogel beads with different functional attributes. Alginate is an anionic block copolymer that is composed of 1–4-linked β -D-mannuronic acid and α -L-guluronic acid units, with the negative charge originating from carboxyl groups (Lee & Mooney, 2012). Carrageenan is an anionic polymer that is composed of galactan monomers linked together by alternating (1 $\rightarrow$ 3)- and (1 $\rightarrow$ 4)- β -d-glycosidic bonds with the negative charge originating from sulfate groups (Li, Ni, Shao, & Mao, 2014). We hypothesized that the hydrogel beads formed by these two different polysaccharides would have different structural and physicochemical properties, which would result in differences in the gastrointestinal fate of the encapsulated curcumin. The potential gastrointestinal fate of the different curcumin delivery systems was studied using a simulated GIT (mouth,

stomach, and small intestine). In particular, the influence of the different hydrogel beads on the bioaccessibility of encapsulated curcumin was compared. The information obtained in this study may facilitate the rational design of hydrogel beads to modulate the bioavailability of encapsulated lipophilic nutraceuticals.

2. Materials and methods

2.1 Materials

Corn oil was purchased from a local supermarket and used without further purification. The following chemicals were purchased from the Sigma Chemical Company (St. Louis, MO): alginic acid (sodium salt) (Lot# 180947, viscosity of 1% alginic acid in water is 15–20 mPa s); curcumin (C1386-10G, purity ≥ 65%); mucin from porcine stomach (M2378-100G); pepsin from porcine gastric mucosa (P7000-25G); lipase from porcine pancreas pancreatin (L3126-100G); porcine bile extract (B8631-100G); Tween 80 (P1754-1L); and Nile Red (N3013-100MG). Carrageenan was kindly donated by FMC Biopolymer (Viscarin SD 389, Philadelphia, PA). All chemicals used were analytical grade. Double distilled water was used to make all solutions.

2.2. Preparation of oil-in-water emulsions

Initially, an aqueous phase was prepared by mixing 1% (w/w) Tween 80 with a buffer solution (5 mM phosphate buffer saline (PBS), pH 6.5) and stirring for at least 2 h. The emulsifier solution were then stored overnight at 4 °C to ensure complete dissolution. Excipient emulsions stabilized by Tween 80 were prepared by homogenizing 10% (w/w) corn oil with 90% (w/w) aqueous emulsifier solution using a high-speed blender for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). The droplet size in this coarse emulsion was reduced by passing it through a high-pressure homogenizer (Microfluidizer, M110Y, Microfluidics, Newton, MA) with a 75-μm interaction chamber (F20Y) at an operational pressure of 12,000 psi (82.7 MPa) for 3 passes. The resulting excipient emulsions were stored in a refrigerator at 4 °C before use.

2.3. Preparation of curcumin-loaded emulsion

The curcumin was solubilized within the lipid phase of the emulsions using a

method described previously (Zou, et al., 2015). Curcumin (9 mg) was weighed into a beaker and then excipient emulsion (10% oil, 30 mL) was added, and then held at 100 °C for 10 min. This procedure ensures that the curcumin powder fully dissolves in the lipid phase, without leading to appreciable loss of the curcumin. This amount of emulsion was utilized because it has the ability to completely dissolve the curcumin based on the measured equilibrium solubility of curcumin reported in corn oil at ambient temperature $\approx 3.2 \pm 0.1$ mg/mL (Ahmed, et al., 2012). The emulsion contained 10% corn oil and can therefore dissolve about a 10-times lower amount of curcumin than the amount dissolved in pure corn oil. The resulting curcumin-loaded emulsion was stored in the refrigerator (4°C) prior to hydrogel bead fabrication.

2.4. Encapsulation of curcumin in carrageenan and alginate beads

Aqueous κ -carrageenan and alginate (2.5% w/w) solutions were prepared by dissolving powdered polysaccharide ingredients in distilled water and then stirring continuously at 60 °C for an hour, and then reducing the temperature to 35 °C. Polysaccharide solutions and curcumin-loaded emulsions were then mixed together (1:4, mass ratio) for 2 h with continuous stirring to form a solution that contained 8% emulsion (w/w) and 0.5 % polysaccharide (w/w). Curcumin-loaded carrageenan hydrogel beads were prepared using a syringe to drip curcumin-loaded emulsion/ κ -carrageenan mixtures into 10 mL 5% potassium chloride solution with continuous stirring. Similarly, curcumin-loaded alginate hydrogel beads were formed by dripping curcumin-loaded emulsion/alginate mixtures into 5% calcium chloride solution. The hydrogel beads formed were incubated with the cations (K^+ or Ca^{2+}) for a 1 h at ambient temperature to promote cross-linking. The beads were then collected by filtration and subsequently washed with distilled water and phosphate buffer to remove any excess ions from their surfaces.

2.5. Gastrointestinal tract model

Curcumin delivery systems with the same total lipid concentration (5%) and total volume (15 mL) were prepared by diluting the original systems with buffer solution (5 mM PBS, pH 6.5). The samples were then passed through a static GIT model that simulated the mouth, stomach, and small intestine phases. This method has been

described in detail in our previous study(Zhang, Zhang, Zhang, Decker, & McClements, 2015a), and so is only briefly summarized below.

Initial System: 15mL of the initial systems were placed into a glass beaker in an incubator shaker at a rotation speed of 100 rpm for 15 min at 37 °C for preheating (Innova Incubator Shaker, Model 4080, New Brunswick Scientific, Edison, NJ). Three different initial curcumin-loaded delivery systems were tested: (i) lipid droplets, (ii) filled alginate beads, and, (iii) carrageenan beads.

Mouth phase: 15 mL of simulated saliva fluid (SSF) containing 0.03 g/mL mucin was preheated to 37 °C and then mixed with the initial samples. After being adjusted to pH 6.8, the mixture was incubated in an incubator shaker for 10 min at 37 °C to mimic agitation in the mouth.

Stomach phase: 30 mL of the “bolus” sample resulting from the mouth phase was mixed with 30 mL of simulated gastric fluid containing 0.0032 g/mL pepsin preheated to 37 °C and then the pH was adjusted to 2.5. This mixture was incubated in the incubator shaker for 2 h at 37 °C to mimic stomach conditions.

Small intestine phase: 60 mL of “chyme” sample from the stomach phase was placed into a 150 mL glass beaker that was placed into a water bath at 37 °C and then adjusted to pH 7.00. 3 mL of simulated intestinal fluid was added to the reaction vessel, followed by 7 mL of bile salt solution with constant stirring. The pH of the reaction system was adjusted back to 7.00. 5 mL of lipase solution was then added to the sample and an automatic titration unit (Metrohm, USA Inc.) was used to monitor the pH and maintain it at pH 7.0 by titrating 0.25 mM NaOH solution into the reaction vessel for 2 h at 37 °C. The amount of free fatty acids released was calculated from the titration curves as described previously(Patel, Bouwens, & Velikov, 2010).

2.6. Determination of particle characterization

The particle size distribution of the samples was measured using a static light scattering device (Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK).Initial, mouth, and small intestine samples were diluted with phosphate buffer (5 mM, pH 7.0) and stomach samples were diluted with acidified water (pH 2.5) to avoid

multiple scattering effects. The refractive index of the oil phase used in the calculations was 1.472. The average particle sizes are reported as the surface-weighted mean diameter (d_{32}). The hydrogel beads could not be analyzed by static light scattering because they were too large and rapidly sedimented or creamed inside the measurement chamber. We therefore used image analysis software (Image J, version 1.49) to characterize the particle size distribution of the hydrogel beads based on images of the samples. The images of the hydrogel beads after each digestion stage were obtained using a conventional digital camera, with at least 20 samples being used to determine their particle size distributions.

2.7. Microstructure analysis

The microstructure of the samples was determined after passing through each digestion stage. The microstructure of all the samples was examined using confocal scanning laser microscopy with a 60 × objective lens (Nikon D-Eclipse C1 80i, Nikon, Melville, NY, U.S.). For the confocal microscopy measurements, each sample was dyed prior to confocal microscopy observation. The corn oil was dyed with Nile red solution (1 mg/mL ethanol) by adding 0.1 mL of Nile red dye solution to 2 mL of sample and storing at 5 °C overnight. The excitation and emission spectrum for Nile red were 543 nm and 605 nm, respectively. The microstructure images for confocal microscopy were taken and analyzed using image analysis software (NIS-Elements, Nikon, Melville, NY).

2.8. Determination of curcumin concentration, bioaccessibility, and transformation

After the samples had passed through the simulated GIT, 25g of digesta from the small intestine phase was collected and centrifuged (18,000 rpm, Thermo Scientific, Waltham, MA) at 25 °C for 30 min. The clear supernatant was collected and assumed to be the “micelle fraction” in which the curcumin was solubilized. Aliquots of 3 mL of micelle fraction were mixed with 3 mL of chloroform, vortexed, and centrifuged at 1,750 rpm for 10 min at ambient temperature. The bottom layer containing the solubilized curcumin was collected, while the top layer was mixed with an additional 3 mL of chloroform and the same procedure was repeated. The two collected chloroform layers were mixed together and then diluted to an appropriate concentration to be analyzed by a UV-visible

spectrophotometer at 419 nm. The concentration of curcumin was calculated from the absorbance using a standard curve.

The *bioaccessibility* of curcumin (*i.e.*, the fraction in the small intestine that was solubilized in the mixed micelle phase) was calculated from this data: $B^* = 100 \times C_{\text{Micelle}}/C_{\text{Digesta}}$, where C_{Micelle} and C_{Digesta} are the curcumin concentrations in the micelle fraction and in the total digesta collected after the small intestine phase, respectively. The *transformation* of curcumin (*i.e.*, the fraction remaining in a non-transformed state in the small intestine) was calculated: $T^* = 100 \times C_{\text{Digesta}}/C_{\text{Initial}}$, where C_{Initial} is the curcumin concentration in the small intestine phase calculated based on the initial amount added, which was calculated based on the curcumin concentration in the initial sample before digestion taking into account the dilution steps at each stage of the gastrointestinal model. The *effective bioavailability* (*i.e.*, the fraction of ingested curcumin present in the mixed micelle phase) was also calculated: $BA = 100 \times C_{\text{Micelle}}/C_{\text{Initial}}$. The bioaccessibility and transformation of curcumin in the filled hydrogel beads could not be calculated because it was not possible to extract all of the curcumin from the hydrogel beads, so C_{Digesta} could not be measured.

2.9. Statistical analysis

All experiments were performed on at least two freshly prepared samples. The results are reported as averages and standard deviations, and the differences among treatments were calculated based on an analysis of variance (ANOVA) and a post-hoc Duncan test with a confidence level of 95 %. These analyses were carried out using statistical analysis software (SPSS, IBM Corporation, Armonk, NY, USA).

3. Results and Discussion

3.1 Preparation of the curcumin-loaded emulsions and filled hydrogel beads

In this study, three different curcumin delivery systems were prepared: lipid droplets; filled alginate beads; and filled carrageenan beads (Fig.1). The filled hydrogel beads were formed by injection of a mixture of the lipid droplets and anionic biopolymer into a crosslinking cationic mineral solution. In general, several parameters may influence the

physicochemical and structural properties (*e.g.*, size, shape, porosity, and hardness) of hydrogel beads fabricated by the injection method, including biopolymer concentration, cation concentration, needle diameter, injection flow rate, and hardening time. After some preliminary experiments to optimize the preparation conditions, we fabricated the hydrogel beads by injecting a 0.5 % polysaccharide solution (alginate or carrageenan) into a 5% cationic mineral solution (Ca^{2+} or K^{+}) using a syringe with a needle diameter of 0.5 mm at a rate of one drop per 2 s. After injection, the hydrogel beads were incubated in the cationic mineral solutions for an hour at ambient temperature to promote polysaccharide cross-linking. The alginate beads fabricated using this method had a spherical shape with a smooth surface, and had diameters ranging from about 2 to 3 mm (Fig.1 and Table1). Conversely, the carrageenan beads formed using the same method had an irregular shape and had rough surfaces with a larger mean particle diameter (around 3 to 4 mm). The difference in the properties of the hydrogel beads can be attributed to differences in the gelation mechanisms of the two different polysaccharides. The hydrogel matrix within the alginate beads is formed by calcium ion crosslinking of guluronic acid blocks within the alginate chains (Sikorski, Mo, Skjåk-Bræk, & Stokke, 2007), whereas that in the carrageenan beads is formed by potassium ion crosslinking of sulfate groups along the carrageenan chains (Ellis & Jacquier, 2009). The fact that the alginate beads had a more spherical and smoother structure suggests that the crosslinking of the alginate molecules by calcium ions occurred more rapidly than the crosslinking of the carrageenan molecules by potassium ions. Thus, the initial spherical shape of the polysaccharide solution injected into the cation solution was maintained more readily for the fast-gelling alginate system. This may have been because the multivalent calcium ions were more efficient at crosslinking than the monovalent potassium ions. We hypothesized that the difference in initial structure and properties of the hydrogel beads formed by the two different polysaccharides would alter their behavior within the simulated GIT, *e.g.*, their susceptibility to enzyme penetration, and their stability to mechanical forces or pH changes. In future studies, it would be useful to carry out a more detailed analysis of the structural and physicochemical properties of the different hydrogels, such as the nature of the cross-links formed, the effective pore size of the hydrogel network, and the rheological properties of the beads.

It is interesting to note that unfilled alginate and carrageenan beads sediment to the bottom of the test tubes, whereas the filled beads cream to the top (Fig. 1). This difference in behavior can be attributed to the influence of lipid droplets on the effective density of the hydrogel beads (Matalanis & McClements, 2013): unfilled beads are denser than water, whereas filled beads are less dense than water. This effect occurs because lipids have a lower density than water, whereas biopolymers have a higher density. The buoyancy characteristics of filled hydrogel beads makes them good candidates for stomach targeting delivery systems as they may spend a longer time within the gastric cavity (Murata, Sasaki, Miyamoto, & Kawashima, 2000).

3.2 Influence of GIT conditions on particle size and microstructure

The particle size distribution (Fig. 2), general appearance (Fig. 3), and microstructure (Fig. 4) of the curcumin-loaded lipid droplets and filled hydrogel beads were measured during passage through the simulated GIT.

Curcumin-loaded lipid droplets. The mean particle diameters (d_{32}) of the curcumin-loaded lipid droplets were fairly similar for the initial samples (0.13 μm) and the samples that had been exposed to the mouth (0.14 μm) and stomach (0.15 μm) phases (Table 1). In addition, the particle size distributions of the initial, mouth, and stomach samples were also fairly similar (Fig. 2). These results suggest that the lipid droplets were stable to coalescence in the simulated saliva and gastric fluids, which can be attributed to the fact that they were coated with a non-ionic surfactant (Tween 80) that inhibits droplet coalescence through steric repulsion. Other studies have also reported the good stability of lipid droplets coated by non-ionic surfactants to droplet coalescence under simulated mouth and stomach conditions, i.e., there is little increase in the dimensions of the individual lipid droplets (Mayer, Weiss, & McClements, 2013; Zhang, et al., 2015a). Despite the fact little droplet coalescence was observed by light scattering, the confocal microscopy images indicated that there was some aggregation of the lipid droplets within the mouth and stomach phases (Fig. 4), which may have been due to depletion flocculation caused by the presence of non-adsorbed mucin molecules in the simulated saliva (Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005). These non-adsorbed mucin

molecules generate an osmotic attraction between the lipid droplets that forces them together. These large particles were not seen in the particle size distributions because the samples had to be diluted for the light scattering measurements, which would have caused dissociation of any flocs held together by depletion forces. After passing through the small intestine phase, the particle size distribution of the samples initially containing curcumin-loaded lipid droplets became bimodal (Fig. 2a). This suggests that there was a wide range of different-sized colloidal particles present in the digesta after exposure to small intestinal fluids containing lipase and bile salts. These colloidal particles may have been mixed micelles (micelles and vesicles), undigested lipid droplets, and/or insoluble calcium salts.

Filled hydrogel beads. The particle size and microstructure of the filled hydrogel beads in the different regions of the GIT depended on the type of polysaccharide used (Figs. 2 to 4). For the alginate beads, the general shape of the particle size distribution remained fairly similar from initial-to-mouth-to-stomach, exhibiting a major population of large particles with diameters around 2,500 μm (Table 1, Fig. 2c). In addition, observation of the general appearance of the alginate beads indicated that they remained intact after exposure to the different GIT phases (Fig. 3). There was a slight decrease in the mean particle diameter of the alginate beads when they were transferred from the mouth phase to the stomach phase (Table 1). Previous studies have also reported that calcium alginate beads shrink under highly acidic gastric conditions (Hebrard, Hoffart, Cardot, Subirade, & Beyssac, 2013; Li, Hu, Du, Xiao, & McClements, 2011), which can be attributed to a reduction in the electrostatic repulsion between the alginate molecules below the pK_a value of the carboxyl groups. The closer packing of the biopolymer molecules within the hydrogel beads under stomach conditions may be useful for protecting encapsulated components from the relatively harsh gastric environment. There was a further decrease in the mean diameter of the alginate beads when they were transferred from the stomach phase to the intestine phase (Table 1). Interestingly, there appeared to be an increase in the turbidity of the aqueous solution surrounding the hydrogel beads in the small intestine (Fig. 3b), which suggests that there were colloidal particles present large enough to scatter light. These particles may have been undigested lipid droplets or mixed micelles

released from the hydrogel beads. Indeed, confocal microscopy images of the aqueous phase collected from outside of the hydrogel beads indicated that they contained some lipid-rich particles (data not shown).

The initial mean diameter of the carrageenan beads ($\approx 3,100\mu\text{m}$) was considerably larger than that of the alginate beads ($\approx 2,600\mu\text{m}$), despite the fact that they were fabricated using the same injection method, which may have been due to differences in the initial rate of polysaccharide gelation discussed in the previous section. The dimensions and morphology of the carrageenan beads also appeared to be much more sensitive to the GIT region than the alginate beads (Table 1 and Fig. 3). After exposure to simulated oral conditions, the mean particle diameter of the carrageenan beads decreased appreciably, which appeared to be due to disintegration of some of the larger particles (Figs. 2b and 3b). Particle disintegration may have occurred due to dilution, an increase in ionic strength, or application of mechanical stresses within the simulated oral environment. Alternatively, the anionic mucin molecules may have competed with the anionic carrageenan molecules for potassium ions, which caused disintegration of the carrageenan hydrogels. There was a further reduction in mean particle diameter when the carrageenan beads were exposed to the simulated stomach phase, which may have been induced by changes in pH, ionic strength, or mechanical agitation in the gastric fluids. For example, a change in electrostatic interactions may have promoted hydrogel shrinkage or disintegration. Visual observation of the samples after exposure to the gastric phase indicated that they contained a mixture of large and small irregular shaped particles (Fig. 3b). After exposure to the small intestine conditions, there was an increase in mean particle diameter (Table 1), but visual observation clearly showed that most of the larger particles from the stomach had either been disrupted or had shrank in the intestinal fluids (Fig. 3b). It therefore appeared that the carrageenan beads were much less stable to GIT conditions than the alginate beads. As with the alginate beads, there appeared to be an increase in turbidity of the aqueous phase surrounding the beads in the small intestine, which suggests that lipid droplets or mixed micelles were present.

3.3 Influence of delivery system type on lipid digestion

An automatic titration (pH stat) method was used to evaluate the effect of delivery system type on the rate and extent of lipid digestion. The fraction of free fatty acids (FFAs) released from the samples during the small intestine phase was calculated from measurements of the volume of NaOH that had to be titrated into the reaction chamber to maintain a constant pH (7.0). The nature of the delivery system clearly had a pronounced impact on the rate and extent of lipid digestion (Fig. 5). The free lipid droplets were rapidly digested during the first 10 minutes with over 60% of the FFAs being released, followed by a more gradual digestion for the remainder of the incubation time. Conversely, the rate of FFAs released was much slower for the filled hydrogel beads, and depended on polysaccharide type. For the filled alginate beads, no lipid digestion occurred during the first 30 minutes, then there was a relatively slow rate of lipid digestion at longer times, with only 33% of the FFAs being released by the end of the 2 hour digestion period. For the filled carrageenan beads, lipid digestion started immediately and occurred throughout the incubation period, but it was relatively slow compared to the free lipid droplets, with only 46% of FFAs released by the end of the small intestine phase. These results indicate that encapsulation of the lipid droplets within the hydrogel beads retarded lipid digestion, with the degree of lipase inhibition depending on hydrogel type. Similar results have been reported in earlier studies of lipid digestion in hydrogel beads (Li, et al., 2011).

There are a number of reasons that encapsulation may have inhibited lipid digestion. Firstly, lipid digestion can only occur if pancreatic lipase is able to adsorb to the surfaces of the lipid droplets and come into close proximity with its substrate, *i.e.*, triacylglycerol and diacylglycerol molecules (Reis, Holmberg, Watzke, Leser, & Miller, 2009). For the free lipid droplets, the pancreatic lipase is able to rapidly move from the aqueous phase to the lipid droplet surfaces and initiate lipolysis. For the filled hydrogel beads, lipase and the other GIT components required for digestion (such as co-lipase and calcium) have to penetrate into the beads and diffuse through the hydrogel matrix before they can reach the lipid droplet surfaces. *In particular, these components must diffuse through the pores between the network of cross-linked biopolymer molecules that makes up the interior of the hydrogel beads* (Lee, et al., 2012; Li, et al., 2011). The time taken for lipase molecules to reach the lipid droplets within the interior of the beads increases

as the pore size of the hydrogel matrix decreases, the diameter of the beads increases, or the surface area of the beads decreases(Chan & Neufeld, 2009; Li, et al., 2011). The lag time observed before any lipid digestion occurred for the alginate beads may have been because the hydrogel matrix shrank under gastric conditions thereby decreasing the bead pore size and inhibiting penetration of lipase. However, the pore size expanded once the beads were placed into the small intestinal fluids, thereby allowing the lipase molecules to penetrate. This hypothesis is supported by the fact that the mean diameter of the alginate beads was smaller in the gastric fluids than in the intestinal fluids (Table 1). After the lag phase, the relatively slow rate of lipid digestion observed in the alginate beads, may have been because they were larger than the carrageenan beads. As mentioned earlier, the alginate beads maintained their general structure throughout the GIT, whereas the carrageenan beads were largely disrupted by the small intestine phase. The slow rate of digestion for the alginate beads may also have been because they had a smaller pore size than the carrageenan beads, which would inhibit lipase diffusion. A second potential reason for retarded lipid digestion in the filled hydrogel beads is that the long chain FFAs generated at the surfaces of the lipid droplets have to be removed otherwise the lipolysis reaction will be retarded(Bauer, Jakob, & Mosenthin, 2005; Dahan & Hoffman, 2008). Typically, FFAs are removed from the droplet surfaces by being incorporated into mixed micelles with bile salts and phospholipids, or by forming insoluble salts with calcium ions(Devraj, et al., 2013). The removal of FFAs away from the lipid droplet surfaces may therefore be limited because bile salts, phospholipids, and calcium cannot easily penetrate through the cross-linked biopolymer network inside the hydrogel beads. In addition, the diffusion of the FFAs themselves out of the hydrogel beads may also be inhibited by the presence of the biopolymer network. These results clearly show that encapsulating lipid droplets in hydrogel beads can modulate the rate and extent of their digestion.

3.4 Influence of delivery system type on curcumin release

The amount of curcumin released into the mixed micelle phase clearly depended on the nature of the delivery system used to encapsulate it: free droplets > filled carrageenan beads > filled alginate beads(Fig. 6). For the free oil droplets, it was possible to calculate

the bioaccessibility (73%), transformation (78%), and effective bioavailability (57%) from the initial, digesta, and micelle curcumin concentrations (Section 2.7). The effective bioavailability of the curcumin in the filled alginate beads (12.5%) and carrageenan beads (25.5%) was also determined. Pure curcumin typically has a very low bioavailability because of its poor chemical stability and low solubility in gastrointestinal fluids (Anand, et al., 2007; Heger, et al., 2014). Our results suggest that encapsulation of curcumin within free lipid droplets led to a relatively high effective bioaccessibility. This enhanced bioavailability can be attributed to two main physicochemical factors. First, when curcumin is present within the interior of the lipid droplets it is protected from chemical degradation induced by aqueous phase components, which leads to a relatively high amount remaining in an active form in the small intestine (high T*). Second, the digestion of the lipid droplets leads to the formation of mixed micelles that can solubilize the hydrophobic curcumin molecules (high B*). Previous studies in our laboratory have also reported that nanoemulsions can increase curcumin bioaccessibility and transformation through these mechanisms when used as either delivery systems (Ahmed, et al., 2012) or excipient systems (Zou, et al., 2015; Zou, et al., 2015).

The relatively low effective bioavailability of the curcumin in the filled hydrogel beads can largely be attributed to the fact that not all of the lipid phase was digested for these systems. Consequently, some of the curcumin remained within the undigested lipid droplets trapped inside the hydrogel beads and was therefore not released. In addition, there will have been less mixed micelles available in the aqueous phase to solubilize any curcumin molecules released: (i) because of the relatively low extent of lipid digestion; and, (ii) because the diffusion of any FFAs and monoacylglycerols formed within the beads may have been hindered by the hydrogel matrix. There appeared to be a good correlation between the extent of lipid digestion at the end of the small intestine phase and curcumin release, *i.e.*, they followed the same trend: free droplets > filled carrageenan beads > filled alginate beads.

Overall, these results have a number of important practical implications. First, it is important to ensure that any hydrogel beads used to encapsulate and protect curcumin within food products do not inhibit its release within the GIT. Otherwise, any potential

health benefits associated with consuming curcumin may not be fully realized. Second, it may be possible to control the release of curcumin in different regions of the GIT by encapsulating it within hydrogel beads. For example, if it was important to release curcumin in the colon then it could be encapsulated within hydrogel beads that retained it in the mouth, stomach, and small intestine, but released it in the colon. This kind of retention and release behavior is based on the fact that dietary fibers are not digested within the upper gastrointestinal tract, but are digested by bacteria in the colon (Chourasia & Jain, 2004; Sinha & Kumria, 2001). Alternatively, if one wanted to mask the taste of curcumin, but still ensure that it was absorbed in the upper intestine, it could be incorporated into hydrogel beads that retained it in the mouth, but released it in the stomach or small intestine. In this case, the hydrogel beads may be designed so that they dissociate or are digested within the stomach or small intestine, which may be triggered by changes in pH, ionic strength, or enzyme activity (Zhang, Zhang, Chen, Tong, & McClements, 2015b).

4. Conclusions

In the present study a simulated GIT was used to study the influence of different types of delivery systems on lipid digestion and curcumin release: free lipid droplets; filled carrageenan beads; and, filled alginate beads. In all systems, the crystal form of curcumin was first dissolved in a digestible lipid phase, which was then converted into an oil-in-water nanoemulsion. In the case of the filled hydrogel beads, the curcumin-loaded lipid droplets were then trapped within a hydrogel matrix using an injection-gelation method. The alginate beads had a uniform spherical shape with diameters ranging from 2 to 3 mm, whereas the carrageenan beads had irregular shapes and diameters around 3 to 4 mm. Differences in hydrogel bead morphology were attributed to differences in the initial rate of biopolymer gelation during the injection process. Simulated GIT studies indicated that free lipid droplets were digested more rapidly and fully than those encapsulated within hydrogel beads. In addition, they showed that the rate and extent of lipid digestion in filled hydrogel beads depended on polysaccharide type, which was partly attributed to differences in their integrity within the GIT. Alginate beads remained relatively intact throughout the GIT, while the

carrageenan beads partly disintegrated. The extent of curcumin release into the mixed micelle phase in different samples followed the following order: free droplets > filled carrageenan beads > filled alginate beads. Our results have important implications for designing hydrogel beads to increase the oral bioavailability of lipophilic bioactives.

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Table 1. The mean particle diameters of three curcumin delivery systems after exposure to different stages of a simulated GIT: free lipid droplets; filled alginate beads; and, filled carrageenan beads.

Sample	Mean Particle Diameter (µm)			
	Initial	Mouth	Stomach	Intestine
Free lipid droplets	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.22 ± 0.01
Filled alginate beads	2604±75	2857 ±166	2555± 204	1796± 58
Filledcarrageenan beads	3107± 159	824± 640	372±344	848±184

Figure 1. Appearances of different curcumin-delivery systems prepared in buffer solution (5mMPBS, pH 6.5). From left to right: free lipid droplets; filled alginate beads, and filled carrageenan beads. Unfilled alginate beads (far right) are shown as a reference. Unfilled carrageenan beads also sank to the bottom of the tubes.

(a)

(b)

Figure 2. Particle size distributions of different samples after exposure to different GIT phases: (a) free lipid droplets, (b) filled carrageenan beads, and (c) filled alginate beads.

(a)

(b)

Figure 3. Images of different curcumin-loaded beads after each digestion stage: (a) filled alginate beads, and (b) filled carrageenan beads.

Figure 4 Microstructures of free lipid droplets, filled alginate beads and filled carrageenan beads exposed to different regions of a simulated GIT. Scale bar is 20 μm .

Figure 5. Amount of free fatty acids released from free lipid droplets, filled alginate beads and filled carrageenan beads measured using a pH-stat *in vitro* digestion model.

Figure 6. Amount of curcumin present in the mixed micelle phase for free lipid droplets, filled alginate beads, and filled carrageenan beads after *in vitro* digestion.

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Graphical Abstract