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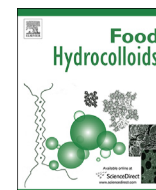

Pickering emulsions immobilized within hydrogel matrix with enhanced resistance against harsh processing conditions and sequential digestion

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Protein particles stabilized Pickering emulsions are unstable under harsh processing conditions and vulnerable to premature lipid phase release during gastric digestion. The objective of this study is to encapsulate kafirin nanoparticles-stabilized Pickering emulsions (KPE) within hydrogel matrix to develop orally administrated Pickering emulsions with enhanced storage and sequential release properties. By premixing KPE and sodium alginate with different volume ratios, emulsion hydrogels (EGs) with various emulsion fractions were immobilized within the calcium ions crosslinked sodium alginate hydrogel matrix thereafter. Ultra small-angle x-ray scattering (USAXS)/small-angle x-ray scattering (SAXS) analyses suggested that encapsulation of KPE resulted in shrinkage in hydrogel network mesh size, and the emulsion interface evolved from smooth to course one as volume ratio of sodium alginate to KPE increased. When incubated under alkaline (i.e., pH = 8.5) or high-temperature (i.e., 60 °C) conditions which were previously reported to cause severe structural collapse for KPE, the coalescence and lipid phase release in EGs were largely inhibited. EGs were also found to be less subjective to stimulated gastric digestion, while the collapse of EGs and thus the release of lipid phase took place in simulated intestinal fluid. The volume fraction of alginate in EGs did not affect the ultimate free fatty acid (FFA) release extent, but it had a negative correlation with the bioaccessibility of lipophilic nutraceutical. This study highlights the potential of designing hydrogel carrier for the oral administration of Pickering emulsion with ease of preparation, improved processing stability and controlled digestion profile.

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1. Introduction

Pickering emulsions, emulsion droplets stabilized by colloidal particles (Pickering, 1907), exhibited peculiar advantages over conventional emulsions in terms of outstanding stability against coalescence and Ostwald ripening (Dickinson, 2010), the capacity of stabilizing emulsions with high internal phase (Ikem, Menner, & Bismarck, 2008) as well as introducing novel rheological (Xiao, Wang, Gonzalez, & Huang, 2016) and thus sensory properties. For these reasons, Pickering emulsion based formulations have gained renewed research interest in fields of food, material, cosmetics and pharmaceutics (Xiao, Li, & Huang, 2016; Tang, Quinlan, & Tam, 2015; Yi, Yang, Jiang, Liu, & Jiang, 2011). Among which, edible particles stabilized Pickering emulsions were expected to function as promising vehicles for encapsulation, protection and delivery of bioactive nutraceuticals through oral administration route (Jia et al., 2015; Wang et al., 2015; Wu et al., 2015). However, formulations that can meet challenges during the processing procedures of food/pharmaceutical products and serve for functional delivery purposes are far from readily available. In our previous research, we established a kafirin protein nanoparticles stabilized Pickering emulsion (Xiao, Li & Huang, 2016; Xiao, Wang, Gonzalez & Huang, 2016). Although it exhibited long-term stability towards coalescence and possessed protection effects towards lipid phase during storage, they could not withstand high temperature and alkaline treatments. And the emulsion droplets lost their structure integrity

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due to proteolysis in gastric digestion fluid (Xiao, Li, & Huang, 2015). Design Pickering emulsion based delivery systems with enhanced resistance against the harsh processing and gastric conditions, so as to deliver active agents to specific locations within gastrointestinal tract, is one of the urgent challenges for edible particles stabilized Pickering emulsions.

Previous research efforts in enhancing stability and controlled release of lipids in conventional emulsion systems involved multilayer emulsion systems via coating lipid droplets with biopolymer coatings (Bortnowska, 2015), emulsion - filled hydrogel beads through physical or chemical crosslinking processes (Chen et al., 2007; Gulsen & Chauhan, 2005; Guo, Ye, Lad, Dalgleish, & Singh, 2014; Li & McClements, 2011; Shingel, Roberge, Zabeida, Robert, & Klemberg-Sapieha, 2009) etc. In these cases, small molecular weight surfactant or surface-active biopolymers in primary emulsions stabilize the non-polar phase and/or function as network forming component.

In this study, we intend to immobilize Pickering emulsion droplets within hydrogel strips using a physical crosslinking method, which may fulfill the application potential of protein particles stabilized Pickering emulsion as an oral drug delivery vehicle with programed release profile. To this end, alginate, an unbranched binary copolymer of β (1/4) linked β -D mannuronic acid (M) and α-L-guluronic acid (G) (Draget, 2009), was selected as the coating layer and hydrogel formation matrix due to its ability to form mechanically consistent hydrogel in the presence of divalent cations as well as evidenced resistance towards gastric digestion (Fajardo et al., 2012; Li, Hu, Du, Xiao, & McClements, 2011; Liu, Liu, Liu, Li, & Liu, 2013). To elucidate the microstructure and digestion profile of as-prepared emulsion hydrogel systems (EGs), curcumin, a lipid-soluble nutraceutical with intrinsic fluorescence (Xiao, Nian, & Huang, 2015), was chosen as a lipophilic encapsulate marker.

The primary Pickering emulsion stabilized by kafirin protein nanoparticles (KPE) was mixed with different volume ratios of sodium alginate solution. The as-prepared mixture was then dripped into 1% $CaCl_2$ solution to induce network formation among Ca^{2+} and anionic G-G rich sequences in sodium alginate. Instead of forming spherical hydrogel beads, which is the most commonly adopted morphology for calcium-alginate hydrogel, we injected the mixture into bulk $CaCl_2$ solution by a pipette in a continuous and fast manner, the resultant emulsion embedded hydrogels (EGs) were thus in a strip form. Fluorescence microscopy as well as the combined ultra-small-angle X-ray scattering and small-angle X-ray scattering (USAXS/SAXS) were carried out to reveal the multilevel microstructure of EGs. The stability of EGs under harsh processing conditions, which were reported to cause structural damage to KPE (Xiao, Li & Huang, 2015), was then evaluated. Release of lipid was evaluated using *in vitro* simulated gastric fluid and simulated small intestine fluid with the presence of pepsin and pancreatin, respectively. The impacts of emulsion hydrogel composition on the extent of lipid digestion and bioaccessibility of curcumin were also examined. Results gained in this work will fill the gap between knowledge in edible particles stabilized Pickering emulsion and their practical applications in oral delivery systems.

2. Materials and methods

2.1. Materials

Kafirin protein with a purity of 90% was extracted from whole sorghum grain and characterized in our lab (Xiao et al., 2015b). Sodium alginate (Fine food & pharmaceutical grade, lot number: IL-1-150, viscosity 20–50 mPa for a 1% solution at 20 °C) was purchased from Kimica Corporation (Chuo-Ku, Tokyo, Japan). Curcumin (82% curcumin, 15% demethoxycurcumin (D-Cur) and 3% bisdemethoxy-curcumin (BD-Cur), Mw 361.05) was a gift from Sabinsa Corporation (Piscataway, NJ, USA) and used without further purification. Pure Wesson vegetable oil (ConAgra Foods. Inc., USA) was purchased from a local market and used without further purification. Glacial acetic acid, analytical grade HCl and NaOH were purchased from Alfa Aesar (Ward Hill, MA). Calcium chloride dehydrate ($CaCl_2 \cdot 2H_2O$), sodium chloride, bile salts, Pepsin from porcine gastric mucosa (P7125, ≥400 units/mg protein), pancreatin with 8 × USP specification, Tris maleate iron (II) chloride, ammonium thiocyanate, cumene hydroperoxide, 2-thiobarbituric acid, 1,1,3,3-tetraethoxypropane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium taurodeoxycholate (Na TDC) was purchased from CalBiochem (La Jolla, CA). Water purified by Milli-Q system was used for sample preparation.

2.2. Pickering emulsion preparation

Curcumin encapsulated KPE were formulated as described in our previous paper (Xiao, Li & Huang, 2015). Briefly, curcumin was dissolved in vegetable oil to the final concentration of 3 mg/mL. Kafirin protein was first dissolved in acetic acid and then added dropwise into bulk water to form nanoparticles suspension, dialysis was then conducted in Spectra/Por® 7 RC dialysis tubes (MWCO: 10 kDa) to remove excess acetic acid. Pickering emulsions (total volume 6 mL) were prepared by homogenizing 3 mL vegetable oil (containing 3 mg/mL curcumin) with 3 mL kafirin particle suspension (1% w/v) in a glass vial (1.3 cm in internal diameter and 6 cm in length) under high-speed homogenizer unit (model IKA-ULTRATURRAX T25 digital, IKA 190 Works, Inc., Wilmington, NC, USA) with a 10 mm dispersion probe at 13, 000 rpm for 3 min. The as-prepared Pickering emulsions showed a wide size distribution range (10–120 μm), and the volume averaged size (D_{43}) was around 45 μm.

2.3. Preparation of Pickering emulsion encapsulated alginate hydrogel

Sodium alginate (1.5 wt%) was dissolved with DI water at 40 °C and then mixed for 4 h under stirring. Subsequently KPE and the sodium alginate solution were mixed at volume ratio of (1:1, 1:2, 1:4, 1:8) under stirring for 5 min. Ultrasonication (FS- 28 solid-state ultrasonicator with sonic power, 225 W; sweep frequency, 40 kHz, Fisher Scientific, Pittsburgh, PA, USA) was applied for 1 min to disrupt any flocculated droplets formed during the mixing process. The mixture was injected manually by a 1 mL pipette tip into bulk $CaCl_2$ solution (1 wt%) in a fast and continuous manner, the tip was immersed in bulk $CaCl_2$ solution when injection took place. The hydrogels were allowed for hardening in $CaCl_2$ solution for anther 30 min before further experiment. The obtained hydrogels were named as EG11, EG12, EG14, EG18, respectively. Sodium alginate based hydrogel without KPE encapsulation was prepared parallel as a control and abbreviated as AG. Experiments were performed in triplicate for each formulation.

2.4. Optical microscopy observation

Optical microscopy observation of emulsions was visualized using a Nikon Eclipse TE 2000-U with a Q-Imaging camera. Emulsions or emulsion hydrogels were deposited onto a concave glass slice and covered by a coverslip before imaging at 100 magnification under bright and fluorescence fields. The droplet size of emulsion was analyzed via Image J2x 2.1.4.7 by measuring a minimum of 50 droplets for each sample. The volume averaged emulsion droplet size (D_{43}) was calculated by equation: $D_{43} = \frac{\sum_1^n D_i^4}{\sum_1^n D_i^3}$.

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