



Effects of environmental factors on the physical stability of pickering-emulsions stabilized by chitosan particles



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ABSTRACT

Oil-in-water (O/W) Pickering emulsions stabilized by food-grade particles have gained increasing research attention recently. The aim of the present study was to investigate the effects of chitosan concentration and environmental factors i.e., ionic strength, temperature, and pH on the physical stability of the emulsions stabilized by self-aggregated chitosan particles. The emulsions were prepared using a high speed homogenizer and their coalescence, creaming, and flocculation stability was evaluated. Increasing the chitosan concentration from 0.01 to 0.3% w/v was found to enhance the coalescence and creaming stability. Similar results were obtained with the addition of NaCl from 100 to 500 mM. The emulsions were also highly stable at pH 7 and above. Reducing the pH was found to promote droplets coalescence and creaming, with demulsification occurring at pH 2. The emulsions were stable at and below 50 °C. Increasing the temperature was found to promote droplets coalescence and creaming. The improvement in the emulsion stability could be attributed to the aggregation of particles at the O/W interface and the formation of chitosan networks in the continuous phase, thus limiting droplet–droplet interactions. In conclusion, the physical stability of Pickering emulsions stabilized by chitosan particles was found to be responsive to environmental stimuli. This feature could be exploited in the development of stimulus-responsive emulsions for food or pharmaceutical applications.

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1. Introduction

Since the pioneering work of Pickering more than a century ago (Pickering, 1907), the research on emulsions stabilized by colloidal particles i.e., Pickering emulsions, has received considerable attention in recent times (Berton-Carabin & Schroen, 2015; Ho et al., 2016). More importantly, Pickering emulsions stabilized by nano- and microparticles derived from polysaccharides such as chitin (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011; Zhang et al., 2015), starch (Song et al., 2015), and cellulose (Jia et al., 2015), have ignited an unparalleled interest in both industrial and academic research settings especially because of their potential utilization in food and pharmaceutical applications.

Recently, self-aggregated chitosan particles were used to form O/W emulsions (Ho et al., 2016; Liu, Wang, Zou, Wei, & Tong, 2012), which can then be used to fabricate other hierarchical structures

(Mwangi, Ho, Ooi, Tey, & Chan, 2016; Wei, Wang, Zou, Liu, & Tong, 2012). Chitosan is an amine-rich polysaccharide consisting of repeating D-glucosamine and N-acetyl-D-glucosamine units linked via $\beta(1 \rightarrow 4)$ glycosidic bonds. It is obtained from alkaline deacetylation of chitin that is found in the exoskeletons of crustaceans and the cell walls of certain algae and fungi (Mohammed, Williams, & Tverezovskaya, 2013). The pK_a value of the amino groups of D-glucosamine units tends to 6.5 when the degree of deacetylation is greater than 75% (Koukaras, Papadimitriou, Bikiaris, & Froudakis, 2012) and therefore chitosan undergoes a sol–gel transition in aqueous media. More importantly, the aggregates formed at $pH > pK_a$ have been found to have higher affinity to oil as result of enhanced hydrophobicity (Ho et al., 2016). Owing to its non-toxicity and potential health benefits, chitosan has been permitted in many countries for use as a food additive (JFCRF, 2014), food processing aid (EU, 2011; FDA, 2011; FSANZ, 2013), and dietary supplement (EU, 2012; FSANZ, 2013).

In the past, chitosan has been used to form and stabilize emulsions by increasing the viscosity of the continuous phase (Payet & Terentjev, 2008) or by coating emulsion droplets stabilized by proteins (Hong & McClements, 2007), anionic surfactants

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(Ogawa, Decker, & McClements, 2003; Sunintaboon et al., 2012), nonionic surfactants (Klinkesorn & Namatsila, 2009), or polysaccharides (Aoki, Decker, & McClements, 2005). However, the understanding on how emulsions stabilized by chitosan particles may respond to the environmental changes is still limited. This understanding is useful for predicting product stability, or for creating novel food ingredients that are responsive to environmental stimulus.

In the present study, we evaluated the effects of chitosan concentration and environmental factors viz., ionic strength, pH, and thermal treatment on the properties of emulsions stabilized by chitosan particles. Chitosan particles were characterized using dynamic light scattering, laser Doppler micro-electrophoresis, and spectrophotometric techniques whereas the emulsions were studied using laser diffraction, optical microscopy, and confocal laser scanning microscopy.

2. Materials and methods

2.1. Materials

Chitosan powder with a degree of deacetylation of 75–85% and molecular weight of 50–190 kDa and fluorescein isothiocyanate (FITC) were purchased from Sigma–Aldrich (USA). 2-propanol, glacial acetic acid, and hydrochloric acid were obtained from Friendemann Schmidt Chemical (Australia) while acetone, sodium hydroxide, sodium carbonate, sodium hydrogen carbonate, and dimethyl sulfoxide (DMSO) were acquired from Fisher Chemical (UK). Medium chain triglycerides (MCT) were obtained from KLK Oleo (Malaysia). The physicochemical properties of MCT are shown in Table 1. Ultrapure water with a resistivity of 18.2 MΩ.cm, from a water purification system (Direct-Q 8 UV, Merck Millipore, Darmstadt, Germany), was used throughout the study.

2.2. Preparation of chitosan particles

Chitosan particles were synthesized by a method previously described by Mwangi et al. (2016). Briefly, glacial acetic acid was added into ultrapure water to make acetic acid solution. While stirring at room temperature, 0.01, 0.1, 0.2, or 0.3% (w/v) chitosan was added into the acetic acid solution and left to stir overnight. The mass ratio between chitosan and acetic acid was fixed at 2:3. The particles were formed after adjusting the pH value of chitosan solution from 3.8 to 6.7 using 0.1 M NaOH for 0.01% (w/v) chitosan solution and 4 M NaOH for 0.1, 0.2, and 0.3% (w/v) chitosan solutions.

2.3. Characterization of chitosan particles

The formation of chitosan particles as a result of self-agglomeration of chitosan molecules was monitored by turbidity, particle diameter, and zeta potential measurements. The effect of environmental factors viz., ionic strength (0–500 mM),

temperature (20–90 °C), and pH (2–8), on the turbidity, particle diameter, and zeta potential of chitosan dispersions (0.1% chitosan concentration) was also investigated. The turbidity profile was obtained using a UV–visible spectrophotometer (UV-Genesys 10 UV, Thermo Electron Corporation, USA) at 600 nm. The diameter and zeta potential of the particles were determined by dynamic light scattering and laser Doppler micro-electrophoresis techniques, respectively (Zetasizer Nano-ZS, Malvern Instruments, UK). To avoid multiple particle scattering effects, the samples were diluted to 0.1 mg/ml using ultrapure water.

2.4. Measurement of interfacial tension

In order to get some insight on the surface activity and the mechanism for emulsion stabilization, we measured the interfacial tensions of chitosan dispersions using a force tensiometer (Sigma 702, Dyne Testing, UK) at 25 °C. Before every measurement, a platinum du Noüy ring was rinsed with ultrapure water, 2-propanol, and acetone and then heated in a gas burner flame. After cooling, the ring was hung on a hook on the tensiometer and then submerged into the 'heavy phase'. With the aid of the OneAttention software, the maximum force exerted by the meniscus was used to calculate the interfacial tension using the Huh-Mason correction.

2.5. Preparation of pickering emulsions

Twenty per cent (v/v) of MCT was slowly added to a dispersion of particles (0.01, 0.1, 0.2, or 0.3% w/v chitosan concentrations) in a glass vial while mixing at 10,000 rpm with a high speed homogenizer (IKA-Ultra-Turrax T25 basic, IKA-Werke, Germany). After addition of all the oil, the sample was mixed for an additional 3 min.

In order to study the effect of ionic strength on the properties of the emulsions, an appropriate amount of NaCl powder was directly added to 20 ml of freshly prepared emulsions (0.1% chitosan concentration and at 20% oil loading) to a concentration of 0–500 mM. The samples were gently stirred for 5 min and then were left to stand at room temperature for 7 days before analysis.

In addition, the effect of pH on properties of emulsions was studied. Briefly, the pH of the continuous phases of 20 ml of the freshly prepared emulsions (0.1% chitosan concentration and at 20% oil loading) was adjusted to 2, 3, 4, 5, 6, 7, or 8 using either 1 M NaOH or 1 M HCl. The samples were then left to stand at room temperature for 7 days before analysis.

Twenty milliliters of the freshly prepared emulsions (0.1% chitosan concentration and at 20% oil loading) were also incubated at fixed temperatures (20–90 °C) for 30 min to investigate effect of thermal processing. The samples were then left to stand at room temperature for 7 days prior to analysis.

2.6. Characterization of pickering emulsions

The volume-weighted mean diameter, $D[4,3]$ (Equation (1)), and

Table 1
Physicochemical properties of medium chain triglycerides (MCT).

Viscosity (mPa.s) ^a	Density (g/cm ³) ^a	Surface tension (mN/m) ^b	Interfacial tension (mN/m) ^b			Fatty acid (FA) content (w/w%) ^a
			Ultrapure water	CSS pH 3.8 0.01 (w/v)	CSP pH 6.7 0.01 (w/v)	
28	0.95	29.49 ± 0.00	17.47 ± 0.36	17.87 ± 0.09	18.24 ± 0.50	C8:0 = 72; C10:0 = 28

CSS = chitosan solution; CSP = chitosan particles' dispersion.

^a Provided by the manufacturer.

^b Measured in our lab.

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