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Influence of buffer on the preparation of multilayered oil-in-water emulsions stabilized by proteins and polysaccharides

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ABSTRACT

This study describes the influence of buffer type and ionic strength on the stability of multilayered emulsions produced by biopolymers, such as fish gelatin, whey protein isolate (WPI) and sugar beet pectin. Primary oil-in-water emulsions (5% (w/w) rapeseed oil, and 0.95% (w/w) fish gelatin or WPI) were prepared in different buffers (5, 10, 25, and 100 mM acetate or citrate buffer, pH 3.5) using a microfluidizer. These emulsions were mixed with aqueous sugar beet pectin solution and an appropriate buffer to yield secondary emulsions (0.5% (w/w) rapeseed oil, 0.095% (w/w) fish gelatin or WPI, and 0.2% (w/w) pectin). Secondary emulsions were then added to fish gelatin and WPI solution, respectively, to produce tertiary emulsions (0.1% (w/w) rapeseed oil, 0.019% (w/w) WPI or fish gelatin, 0.04% (w/w) pectin, and 0.5%, 0.6%, and 1.0% (w/w) WPI or fish gelatin, respectively). The influence of buffer type and ionic strength on the stability of primary, secondary and tertiary emulsions was determined by measuring particle size and ζ-potential, as well as assessing the microstructure by optical microscopy. WPI-stabilized primary emulsions produced in citrate buffer were prone to aggregation, especially at high ionic strengths unlike fish gelatin. Production of stable tertiary WPI-stabilized emulsions was only possible in acetate buffer, regardless of ionic strength. By comparison, triple-layered emulsions prepared in citrate buffer showed extensive aggregation. These findings provide a better understanding of the use of biopolymers to prepare multilayered emulsions.

1. Introduction

Protein-stabilized oil-in-water emulsions are known to have thin, but electrically charged interfacial membranes, hence, their major stabilization mechanism is electrical rather than steric repulsion (McClements, 2004a). The properties and stability of protein-stabilized emulsions largely depend on the magnitude and sign of the electrical charge, which depend on the type and concentration of surface-active molecules present at the interfacial membrane and the surrounding conditions, such as pH or ionic strength (McClements, 2009). They tend to aggregate close to the isoelectric point (p*I*) of the protein stabilizing the emulsions or at high ionic strength levels due to the screening effects of charges (Dickinson, 2010). Many studies are already published showing that electrostatically stabilized oil-in-water emulsions are sensitive to pH, salt, heating, freezing, chilling and dehydration (Aoki, Decker, & McClements, 2005; Demetriades, Coupland, & McClements, 1997a, 1997b). A variety of different strategies have been developed to improve the stability of protein-stabilized emulsions, such as the addition of electrically charged surfactants, addition of chelating agents (e.g., EDTA, citrates, phosphates) to sequester multivalent ions, and sequential adsorption of oppositely charged biopolymers to the particle surface using a layer-by-layer (LbL) electrostatic deposition method (Gu, Decker, & McClements, 2005; Keowmaneechai & McClements, 2002; McClements, 2004a).

The LbL method can be used as a powerful tool to carefully control the interfacial properties of multilayered films, such as charge, thickness, permeability, and composition (Guzey & McClements, 2006). Basically, a charged polyelectrolyte solution is mixed with an oil-in-water emulsion (primary emulsion) having an oppositely charged surface to promote adsorption of the polyelectrolyte to form a multilayered membrane (Decher, 1997; Weiss, Takhistov, & McClements, 2006). The adsorption of sufficiently charged polymer to the particle surfaces causes a reversal of net charges either from positive to negative or vice versa (McClements, 2005). This process can be repeated in order to create secondary (two-layered) emulsions; tertiary (three-layered) emulsions up to six or more layers are possible (Chun, Choi, Min, & Weiss, 2013; Decher, Hong, & Schmitt, 1992). The electrical net charge of multilayer coated particles is determined by the outer layer (McClements, 2005).

The formation and stability of multilayered emulsions can be challenging due to extensive droplet flocculation and aggregation, even under

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conditions where polyelectrolyte saturation should occur (Dickinson & Pawlowsky, 1997; Moreau, Kim, Decker, & McClements, 2003; Ogawa, Decker, & McClements, 2004). Two major mechanisms can be observed when multilayered emulsions are formed: bridging or depletion flocculation. Bridging flocculation occurs at certain biopolymer and droplet concentrations due to charge neutralization and bridging effects; depletion flocculation occurs when non-adsorbed polymer exceeds a certain level (Dickinson, 2009; McClements, 2004a, 2004b). Flocculated particles can be disrupted by additional mechanical agitation, such as ultrasonication, blending or homogenization (Guzey, Kim, & McClements, 2004; Moreau et al., 2003; Ogawa et al., 2004). Moreover, flocculation or aggregation could be prevented by carefully controlling the properties of (a) particles (concentration, radius, charge), (b) polymer (molecular weight, conformation, concentration, charge density), (c) solvent (ionic strength, pH, dielectric constant, quality), and (d) mixing method (order of mixing, mixing speed, flow profile) (Guzey et al., 2004; McClements, 2005). Guzey et al. (2004) demonstrated that stable secondary emulsions could be formed by mixing emulsion droplets and biopolymers at pH values where initially there is no interaction, and then adjusting the pH to values where charges occur oppositely. Polymers and droplets are evenly distributed throughout the continuous phase before adsorption occurs, that should ensure rapid and uniform adsorption and reduce the tendency of aggregation (Guzey & McClements, 2006). Moreover, the order of mixing biopolymer solution and emulsion can strongly affect the tendency of aggregation. The addition of emulsion droplets, for example, into a biopolymer solution under conditions where an attractive interaction between polyelectrolytes and particles prevailed results in less aggregation than vice versa (Guzey & McClements, 2006).

Buffers may play a key role in producing multilayered interfacial membranes. In particular, they are used to control the pH at a nearly constant value when small amounts of acid or base are added to the system — a fact that is important to promote electrostatic deposition between polymers and emulsions droplets. Moreover, solutes and ions are known to affect biopolymer stability, which is described by the Hofmeister phenomena that ranks the salting-out effectiveness of various anions and cations for globular proteins (Baldwin, 1996; Curtis & Lue, 2006; Zhang & Cremer, 2006). Since a comprehensive study on the impact of buffer on the formation and stability of multilayered emulsions has not been conducted, one of the major objectives of this article was, therefore, to identify the major factors that influence flocculation of emulsion droplets under prevailing buffer conditions.

The aim of the study was to evaluate the effect of buffer type and ionic strength that influence the adsorption of biopolymer onto the charged surface of protein-stabilized emulsions to produce stable secondary and tertiary emulsions. Different types of proteins (fish gelatin and WPI) were used to prepare primary emulsions containing small droplets in two different buffer systems: citric acid/sodium citrate and acetic acid/sodium acetate at pH 3.5. Fish gelatin is a random coil biopolymer having a highly dynamic and flexible structure, while WPI has a fairly rigid compact globular structure. Subsequently, sugar beet pectin was added to produce secondary emulsions containing droplets coated by a fish gelatin–pectin or WPI–pectin interfacial membranes. Additionally, WPI and fish gelatin were used to create triple-layered emulsions.

2. Materials and methods

2.1. Materials

Cold water fish-skin gelatin (#049K0050) was purchased from Sigma-Aldrich Co. (Steinheim, Germany). Its average molecular weight and p*I* value were reported to be ca. 60 kDa and pH 6, respectively. Whey protein isolate (#ISO345L) was donated from the Carbery Group (Cork, Ireland). Sugar beet pectin (#1 09 03 135) was donated by Herbstreith & Fox KG (Neuenbürg, Germany). The degree of esterification of the beet pectin was 55%, according to the manufacturer. All biopolymers were used without further purification. Citric acid monohydrate (#409107294, purity ≥ 99.5%), acetic acid glacial (#3738.5, purity 100%), analytical grade hydrochloric acid (HCl), and sodium hydroxide (NaOH) were obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Sodium citrate dihydrate (#26996TH, purity ≥ 99.0%) was purchased from SAFC (St. Louis, MO). Sodium acetate (#K91214768 334, purity ≥ 99.5%) was purchased from Merck KGaA (Darmstadt, Germany). Refined rapeseed oil was obtained from a local supermarket and was used without further purification. Deionized water was used for the preparation of all samples.

2.2. Biopolymer solution preparation

Aqueous emulsifier solutions were prepared by dispersing 1% (w/w) fish gelatin powder or WPI powder into 5, 10, 25, and 100 mM citrate buffer or acetate buffer (pH 3.5), respectively. Sugar beet pectin solutions were prepared by dispersing 2% (w/w) powdered pectin into 5, 10, 25, and 100 mM citrate buffer or acetate buffer (pH 3.5), respectively, followed by stirring overnight at room temperature to ensure complete hydration. The pH of each solution was then adjusted to 3.5 using 0.1 and 1 M HCl and/or 0.1 and 1 M NaOH.

In addition, WPI and fish gelatin solutions (1.0% (w/w), 10 mM citrate buffer) were prepared and then adjusted to pH 3.5–10 using 1 M HCl and/or 1 M NaOH. All polymer solutions were kept for 1 min after reaching the pH value before transferring 5 ml of them into glass test-tubes to analyze their ζ-potential after 24 h.

2.3. Emulsion preparation

Primary oil-in-water emulsions were prepared by homogenizing 5% (w/w) rapeseed oil and 95% (w/w) aqueous emulsifier solution at room temperature. A coarse preemulsion was formed by blending oil and emulsifier solution in a high shear blender (Standard Unit, IKA Werk GmbH, Germany) for 2 min. The premixes were then passed through a microfluidizer (M110-EH-30, Microfluidics International Cooperation, Newton, MA) three times at 10,000 psi. The oil-in-water emulsions were mixed with beet pectin solutions (2.0% (w/w), pH 3.5) and appropriate buffer (pH 3.5) using a vortex to get secondary emulsions with the following compositions: 0.5% (w/w) rapeseed oil, 0.095% (w/w) fish gelatin or WPI and 0.2% (w/w) beet pectin. The secondary emulsions were mixed again with WPI or fish gelatin solutions (2% (w/w), pH 3.5) and appropriate buffer (pH 3.5) using a vortex to yield a series of tertiary emulsions with the following compositions: 0.1% (w/w) rapeseed oil, 0.019% (w/w) fish gelatin or WPI, 0.04% (w/w) beet pectin, and 0.5, 0.6, 1.0% (w/w) WPI or fish gelatin, respectively. To form multilamellar coatings, the emulsions were titrated into the biopolymer solutions in order to avoid droplet aggregation and then mixed for 10 s.

2.4. Particle size determination

Particle size measurements were performed using a dynamic light-scattering instrument (Nano ZS, Malvern Instruments, Malvern, UK). Emulsions were diluted to a droplet concentration of approximately 0.005% (w/w) with an appropriate buffer to prevent multiple scattering effects. This technique determines the particle size from measurements of Brownian motion of the particles using light scattering. The size is then calculated from the diffusion constant using the Einstein equation (Dalgleish & Hallett, 1995). The instrument reports the mean particle diameter (z-average) and the polydispersity index (PDI) ranging from 0 (monodisperse) to 0.50 (very broad distribution).

2.5. ζ-potential measurements

Emulsions or individual protein solutions were diluted to a concentration of approximately 0.005% (w/w) with an appropriate buffer. Diluted emulsions were then loaded into a cuvette of a particle