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Glutaraldehyde induced cross-linking of oppositely charged oil-in-water emulsions

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ABSTRACT

The formation and subsequent chemical cross-linking of heteroaggregates from oppositely charged oil-in-water (O/W) emulsions were investigated. For this purpose, 10–30% (w/w) O/W emulsions ($d_{43} \approx 0.5$ –0.9 μm) were prepared at pH 3 using whey protein isolate (WPI) as positively charged emulsifier and sugar beet pectin or Quillaja saponins as negatively charged ones. The oppositely charged emulsions were then combined at a weight ratio of 1:1 and treated with 0, 50, 250, and 500 mM glutaraldehyde as model cross-linker. Particle sizes, ζ-potentials, confocal light scanning microscopic images, and the rheological behavior of the individual and combined emulsions were analyzed. Although FT-IR measurements indicated that glutaraldehyde was able to cross-link all emulsifiers used, combined emulsions stabilized by Quillaja saponins/whey protein isolate remained relatively unaffected from glutaraldehyde treatment as compared to those prepared with sugar beet pectin and whey protein isolate. When the latter pair was treated with 50–500 mM glutaraldehyde, aggregate sizes (d_{43}) significantly increased 1.6- to 10-fold for 10–30% (w/w) O/W emulsions. Furthermore, significantly higher yield stress values were observed when increasing oil content and glutaraldehyde concentration. Simultaneously, decreased phase angles were observed, also confirming the occurrence of a particulate gel after addition of the cross-linker. The presented study provides new insights into the "cross-linkability" of heteroaggregates by chemical and physical means.

1. Introduction

Combined oppositely charged emulsions, often called heteroaggregates, were most recently proposed to be of use as functional ingredients in pharmaceutical, cosmetic, and food applications. For instance, the food industry is in need of novel approaches to reduce the fat content of foods (Iqbal, Hameed, Baloch, & McClements, 2013), since many consumers relate the consumption of fat-rich high caloric foods with incidences of overweight and obesity (Aranceta, Moreno, Moya, & Anadón, 2009). However, the simple omission of fat as an ingredient severely affects viscosity and creaming stability, ultimately affecting the desired mouthfeel (McClements & Demetriades, 1998). Recently, the combination of oppositely charged emulsion droplets was proposed to represent a new approach to create viscous, creamy systems at lower oil droplet concentrations, because the electrostatic attraction of the droplets leads to the formation of oil droplet assemblies that may be highly viscous if properly produced (Mao & McClements, 2012a). Beyond food applications, such heteroaggregates may also be useful in cosmetic and pharmaceutical applications, when the deposition of functional constituents in separate but proximate compartments is required. Interactions between such constituents in neighboring droplets may be modified by modulating the composition and properties of droplet surfaces. For instance, their interfacial composition, charge density, and size were previously found to have a major impact on the properties of the resulting aggregates (López-López, Schmitt, Moncho-Jordá, & Hidalgo-Álvarez, 2006; Rollié & Sundmacher, 2008). By manipulating attractive and repulsive interactions between oppositely charged droplets, Mao and McClements (2012b) modulated the rheological behavior of such heteroaggregates, ranging from low viscous liquids to highly viscous, paste-like products. Our group recently showed the enormous influence of the used emulsifiers on successful heteroaggregate formation, and as a result developed new criteria for selecting appropriate emulsifiers to successfully form such systems. In addition, solely electrostatically stabilized aggregates were found to be highly sensitive towards dilution and shearing, leading

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to severe losses of functional and rheological properties (Maier, Zeeb, & Weiss, 2014). It was suggested that the application of cross-linking agents, such as food-grade laccase and non-food-grade glutaraldehyde, may allow covalent cross-linking beyond solely electrostatic stabilization of heteroaggregates formed with protein- and polyphenol-rich emulsifiers. Laccase catalyzes the conversion of polyphenols into reactive quinones (Selinheimo, Lampila, Mattinen, & Buchert, 2008), which were hypothesized to show a similar cross-linking activity like the di-carbonyl glutaraldehyde (Maier, Oechsle, & Weiss, 2015). In fact, similar aggregate sizes and rheological behaviors were found when aggregates were treated with equimolar amounts of polyphenols (with laccase) and glutaraldehyde. However, the naturally contained phenols in the emulsifying Quillaja extract as well as the solubility of external phenols were too low for substantial cross-linking of covalently cross-linked three-dimensional heteroaggregate gels (Maier, Oechsle, et al., 2015). Consequently, our group previously proposed glutaraldehyde to serve as a convenient model cross-linker for understanding basic fundamentals of carbonyl-driven covalent cross-linking, however, without providing deeper insights yet.

Therefore, aiming at the production of shear-stable and gel-like heteroaggregates, the main objective of this study was to use glutaraldehyde as model di-carbonyl compound to cross-link heteroaggregates produced with the positively charged (below its pI) whey protein isolate and either the negatively charged Quillaja saponins or sugar beet pectin at pH 3. While sugar beet pectin was previously reported to contain covalently-bound phenolic and protein moieties (Funami et al., 2007) potentially reacting with glutaraldehyde, the surface active components of Quillaja saponins are devoid of such phenolic and proteinaceous moieties (Maier, Conrad, Carle, Weiss, & Schweiggert, 2015). Their cross-linking behavior was investigated by examination of FT-IR spectra, particle sizes, ζ-potentials, confocal light scanning microscopy, and rheological measurements. The obtained results should provide insights into covalent cross-linking of protein-based heteroaggregates using a carbonyl cross-linker, ultimately enabling the production of shear-stable and gel-like heteroaggregates for food, cosmetic, and pharmaceutical applications.

2. Materials and methods

2.1. Materials

Quillaja saponin extract (Andean QDP Ultra Organic) was generously provided by Pera Ingredients (Springe-Eldagsen, Germany), labeling a saponin content of 62.5% (w/w). Whey protein isolate (WPI) BiPRO® was donated by Davisco Foods International (Le Sueur, MN, USA). Sugar beet pectin (degree of esterification: 55%) was provided by Herbstreith & Fox (Neuenbürg, Germany). The fluorescence dye Nile Red was purchased from Sigma–Aldrich (Steinheim, Germany). Glutaraldehyde (50% solution in water) and potassium bromide (KBr) for spectroscopy were purchased from Merck (Darmstadt, Germany). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Carl Roth (Karlsruhe, Germany). The medium chain triglyceride mixture Miglyol 812 N was purchased from Cremer Olio (Hamburg, Germany). Ultrapure water was used for the preparation of all samples.

2.2. Preparation of emulsifier solutions and stock emulsions

Aqueous emulsifier solutions were prepared by dispersing 0.5, 0.75, 1.0, 1.25, or 1.5% (w/w) of Quillaja saponins, sugar beet pectin, or whey protein isolate into ultrapure water. After stirring overnight, HCl and NaOH solutions were used to adjust the pH of the emulsifier solutions to pH 3. Combining the respective emulsifier solution with Miglyol oil at a ratio of 10, 15, 20, 25, and 30% (w/w), a pre-emulsion was formed by homogenization at 24,000 min^{-1} for 3 min, using a high-shear blender (labworld-online, Staufen, Germany). Subsequently, pre-emulsions were homogenized by passing them 3 times at ca. 5000 psi (≙ ca. 345 bar) through a high pressure homogenizer (EmulsiFlex-C3, Avestin, Ottawa, Canada) to form fine-dispersed emulsions. For confocal laser scanning microscopy (CLSM), emulsions were stained by adding 0.005% (w/w) of Nile Red to the Miglyol oil prior to homogenization. All emulsions were stored at room temperature for 24 h prior to adjusting to pH 3 (if required) and subsequent analyses.

2.3. Cross-linking treatments of emulsifier solutions

Emulsifier solutions (45 g) containing 1.5% (w/w) of Quillaja saponins, sugar beet pectin, or whey protein isolate were adjusted to pH 3 and 8, respectively. Subsequently, 5 g glutaraldehyde stock solution (5 M) adjusted to pH 3 and 8 were added to the respective emulsifier solutions at room temperature. Control experiments were performed by adding 5 g water adjusted to pH 3, instead of the cross-linking solution. Solutions were stirred for 24 h and then fully evaporated in a drying cabinet at 45 °C and stored in a desiccator for later Fourier transform infrared (FT-IR) analyses.

2.4. Cross-linking treatments of stock emulsions

After pH and ζ-potential verification of the above mentioned stock emulsions, aliquots of 45 g of each stock emulsion were combined with 5 g glutaraldehyde solution.

Glutaraldehyde was used at pH 3 at various dilutions, resulting in a final glutaraldehyde concentration in the stock emulsion assay of 50, 250, and 500 mM, respectively. Control experiments, in which 5 g water adjusted to pH 3 was added instead of glutaraldehyde, were carried out. After adding glutaraldehyde or water to the different assays, the emulsions were vortexed for 10 s and allowed to stand at room temperature for 24 h before further analyses. All experiments were performed in duplicate.

2.5. Combination and cross-linking of oppositely charged stock emulsions

The formation of heteroaggregates was carried out by combining positively charged whey protein isolate stabilized emulsions with each of the negatively charged ones, using 22.5 g of each stock emulsion. Subsequently, 5 g of the glutaraldehyde solution was added. Glutaraldehyde concentrations, control experiments, mixing and storage procedures were performed as described in Section 2.4.

2.6. Characterization of emulsifier solutions, stock and combined emulsions

2.6.1. ζ-potential measurements

To determine the ζ-potential of droplets and aggregates in single and combined emulsions, a particle electrophoresis instrument (Nano ZS, Malvern Instruments, Malvern, UK) was used. Therefore, ultrapure water of appropriate pH value was used to dilute emulsions and combined emulsions to a droplet concentration of approximately 0.005% (w/w). The ζ-potentials were calculated using the Smoluchowski equation. The values reported represent means ± standard deviation of each three readings of two independently prepared samples.

2.6.2. Particle size analyses

A static light scattering instrument (Horiba LA-950, Retsch