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Understanding how the properties of whey protein stabilized emulsions depend on pH, ionic strength and calcium concentration, by mapping environmental conditions to zeta potential

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ABSTRACT

Surface properties play a key role in determining how colloidal particles interact. In the case of emulsion droplets stabilized with whey protein isolate (WPI) the repulsive interactions are thought to be mostly electrostatic and governed by the environment-modulated zeta potential of the droplet surfaces. By coupling a Gouy-Chapman model of the electrical double layer with the chemical equilibria of both the ionizable moieties on the protein and of the binding of calcium by charged groups, the zeta potential of emulsion droplets as a function of pH, ionic strength and calcium concentration is predicted. Experimental data is shown to fit well to this model. In addition the zeta potential alone is shown to be a good predictor of the macroscopic behavior of the emulsion, as characterized by measurements of the low-stress viscosity.

1. Introduction

Whey protein is often used to emulsify oil droplets in dairy products including beverages and yogurts. These products have an ionic strength of around 80 mM and are typically either neutral (pH 6.5–7, beverages) or acidic (pH of 4, yogurt (Kolars, Levitt, Aouji, & Savaiano, 1984)). In such dairy emulsions the ionic background is known to be a complex mix of ions (Jenness & Koops, 1962) and in particular the calcium activity is known to play a major role in product performance (Deeth & Lewis, 2015; Mckinley, 2005).

The stabilizing effect of whey proteins is predominately electrostatic owing to the small size and high charge density of its individual components (Foegeding, Davis, Doucet, & McGuffey, 2002). The ionization degree and hence the stability and aggregation of whey-protein stabilized emulsions depends strongly on pH, ionic strength, and calcium activity of the bulk (Hunt & Dalgleish, 1994; Ju & Kilara, 1998; Kulmyrzaev, Chanamai, & McClements, 2000; Spiegel & Huss, 2002). In order to produce an emulsion that is stable, it is important to ensure that the pH is far from the isoelectric point (IEP) of the proteins and that the salt concentration is sufficiently low, so that the charges are not significantly screened and the zeta potential (ζ) remains high (Kulmyrzaev & Schubert, 2004; Sun & Gunasekaran, 2009).

The electrostatic interaction between droplets can be predicted from surface potential, ionic strength (a proxy for Debye length) and droplet size. The experimental determination of the surface potential is generally an arduous task but it is well approximated by the more accessible zeta potential. The zeta potential is the electrostatic potential at the slipping plane a few molecules away from the surface (Dalgleish, 1997). The manner in which pH, calcium activity and ionic strength affect the interactions, and hence the stability of protein coated emulsion droplets, has frequently been studied (Dickinson & Golding, 1998; Kulmyrzaev, Sivestre, & McClements, 2000; Sosa-Herrera, Lozano-Esquivel, Ponce de León-Ramírez, & Martínez-Padilla, 2012; Ye & Singh, 2000) but a complete quantitative analysis which captures all of these terms has not been attempted to date.

The major component of whey protein from bovine milk is beta lactoglobulin (βLG), followed by alpha lactalbumin and bovine serum albumin. βLG is a small protein with a molecular weight of 18.5 kDa and a radius of gyration $\approx$ 13.9 Å and has a total net charge of $\approx +10$ to ≈ -20 e per molecule depending on the pH (Morr & Ha, 1993; Nicolai, Britten, & Schmitt, 2011; Roefs & de Kruif, 1999).

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The charge on the surface is determined by the equilibrium charging behavior of the amino acids in the surrounding environment. The hypothesis is that this complex charge behavior can be approximated by three generic equilibriums: the protonation of carboxylic acids and amine groups and an electrostatic interaction between carboxylic acid groups and calcium ions:

$$\begin{aligned} &COOH \leftrightarrow COO^- + H^+ \\ &NH_3^+ \leftrightarrow NH_2 + H^+ \\ &COO^- + Ca^{2+} \leftrightarrow COOCa^+. \end{aligned} \quad (1)$$

Notably, in the absence of calcium there are only two equilibria of concern. The pK_a of carboxylic acids is typically around 5, with values of amines much higher, around 12. The large separation between these values means that the separate contributions of the two types of groups to droplet charging behavior can be easily resolved (the bulk of the charging behavior occurs within a few pH units of the pK_a).

The charging behavior of latex particles, metal oxides, metal sulfides, clay, glass and silica has previously been modeled by combining charging estimates using the pK_a value of ionizable surface groups with the Gouy-Chapman model of the electrical double layer (EDL) (Behrens & Grier, 2001; Hizal & Apak, 2006; Larson & Attard, 2000; Leroy & Revil, 2004; Usui & Healy, 2002). Such an approach has been shown to work well when the absolute value of the zeta potential is less than 40 mV, above which Stern layers must be taken into account (Butt, Graf, & Kappl, 2002). In this paper, firstly the effect on the zeta potential of WPI stabilized emulsions of different concentrations of NaCl and different pH is investigated and modeled using this approach. Subsequently, the effect of calcium is introduced, allowing the prediction of the zeta potential from any combination of ionic strength, calcium activity and pH (within the limits of the Gouy-Chapman model). The DLVO theory of colloidal stability (named after Derjaguin, Landau, Verwey and Overbeek) assumes that the total force between colloidal particles is obtained by adding the van der Waals and electrical double layer forces between them (Cosgrove, 2010). A quantitative appreciation of the EDL in these systems also provides the opportunity to highlight the significance of any non-DLVO forces in the experimental data.

With a complete picture of the surface electrical behavior in hand, macroscopic rheological properties and coagulation rates can be compared in the light of the predicted DLVO forces operating. The rheological properties of protein solutions can be influenced by concentration, temperature, pH, ionic strength, and previous processing treatments (Tung, 1978). Intermolecular interactions between charged protein molecules play a large role in determining the rheological properties in solutions and protein-stabilized emulsions (Goodwin, 1975). The consistency index and the flow behavior index are sensitive to changes in pH and protein concentration (Bazinet, Trigui, & Ippersiel, 2004). Indeed, flow properties and yield stresses of suspensions have previously been related to electrokinetic measurements which are a proxy for zeta potential (Hunter, 1982; Scales, Johnson, Healy, & Kapur, 1998).

2. Materials and methods

2.1. Materials

Whey Protein Isolate 895 (sold as ALACENTM 895) was supplied by Fonterra Co-operative Group Ltd, Auckland, New Zealand. It consists of 76% β-lactoglobulin, 15% α-lactalbumin and 3% bovine serum albumin as determined by the manufacturer. Canola oil was purchased from Davis Trading Co., Palmerston North, New Zealand. All chemicals used were of analytical grade, obtained from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise specified. RO water further purified by a Milli-Q system (Millipore, Bedford, MA, USA) was used exclusively.

2.2. Emulsion preparation

2 wt% WPI was mixed with Milli-Q water and stirred at 50 °C for half an hour. The final pH was measured to be 6.3. Native WPI is almost completely soluble at room temperature from pH 3 to 8 (Damodaran, Parkin, & Fennema, 2007). 20 wt% canola oil was added into the mixture and pre-homogenized at 7000 rev/min for 6 min using an Ultra-Turrax T25 (IKA®-Werke GmbH & Co. KG, Staufen, Germany) to form a coarse emulsion. Subsequently a two-stage high pressure homogenizer (type Panda, Niro Soavi, Parma, Italy) was used to emulsify the sample at a pressure of 150 bar on the first stage and 50 bar on the second stage at ambient room temperature (circa 22 °C). The solution was dialysed to have a well-defined ionic background and to remove unadsorbed protein preventing any depletion forces that may arise. The emulsion was dialysed against water with 0.02 wt% sodium azide to prevent any bacterial growth. The cellulose membrane had a molecular weight cut off of 20 *kDa*. Three solution changes reduced the concentration of unadsorbed material by a factor of 5^3. Large droplets were removed by leaving the emulsion in a narrow graduated cylinder for 24 h and discarding the upper 25% of the sample. The emulsion was stored at a temperature of 10 °C. The emulsion droplet size was measured using a Malvern Mastersizer (Malvern Instruments Ltd) (using a general purpose spherical analysis model with particle refractive index of 1.47 in water with zero light absorption and an obscuration value between 12% to 15%) and showed a distribution peaked at around 2 μm. Fig. 1 shows the particle size distribution before and after the removal of the large droplets.

2.3. Zeta potential measurements

Electrolyte concentration varied between 50 and 150 mM as most dairy products have an ionic strength around 80 mM and these ionic strengths maintain the Gouy Chapman validity with absolute values of the surface potential below |40| mV. 1 L NaCl solutions of 50 mM, 100 mM, and 150 mM ionic strength were first prepared. 0.6 ml of WPI emulsion was added to each solution. The solutions were adjusted to different pH values using HCl or NaOH

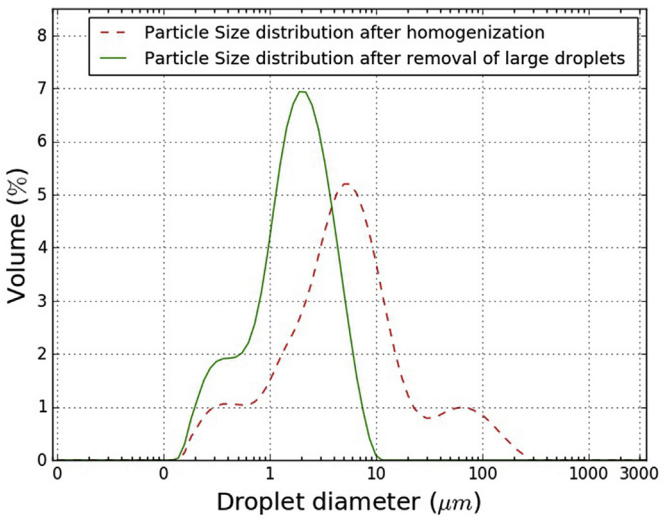

Fig. 1. Droplet diameter distribution before and after the removal of the large droplets measured using a Malvern Mastersizer.

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