Influence of pH and Heating on Physicochemical Properties of Whey Protein-Stabilized Emulsions Containing a Nonionic Surfactant

Kyros Demetriades and D. Julian McClements*

Food Biopolymers and Colloids Research Laboratory, Department of Food Science,
University of Massachusetts, Amherst, Massachusetts 01003

The influence of polyoxyethylene sorbitan monolauarate (Tween 20) on droplet flocculation in 20 wt % soybean oil-in-water emulsions stabilized by whey protein isolate (WPI) was investigated by light scattering, rheology, and creaming measurements. In the absence of Tween 20, emulsions were prone to droplet flocculation near the isoelectric point (pI) of the proteins (4 < pH < 6) but were stable at higher and lower pH values. Flocculation led to an increase in emulsion particle size, viscosity, and creaming index. Addition of Tween 20 at low concentrations (R < 0.1, where R is the surfactant/protein molar ratio) had little effect on emulsion properties, but at higher concentrations (R > 5) it decreased the amount of flocculation and shifted it to lower pH values. The higher concentrations of Tween 20 also reduced the extent of droplet flocculation in emulsions heated at 78 °C for 30 min in the presence of 100 mM NaCl. The results are explained in terms of competitive adsorption, protein–surfactant interactions, and colloidal interactions. This study shows that the physicochemical properties of protein-stabilized emulsions can be controlled by adding nonionic surfactants.

Keywords: *Whey protein isolate; Tween 20; competitive adsorption; pH; emulsions*

INTRODUCTION

Emulsifier interactions are of great scientific and technological importance in the production of ice cream and whipped products (Goff and Jordan, 1989; Gelin et al., 1994; Pelan et al., 1997), as well as during the production, storage, and handling of various other types of food emulsions (Chen and Dickinson, 1995c; Harrison et al., 1995; Nylander and Ericsson, 1997). Industrial food emulsions are complex in both composition and physicochemical properties (Wilde and Clark; 1993; Lucassen-Reynders, 1994; Demetriades and McClements, 1998a,b). The physicochemical properties of emulsions are usually determined by the type and concentration of emulsifier present, environmental conditions (pH and ionic strength), processing (temperature and mechanical agitation), and ingredient interactions (Dickinson, 1997; Hunt and Dalgleish, 1996; Demetriades et al., 1997a,b; Demetriades and McClements, 1998c).

The type of emulsifier present at the droplet interfaces determines the colloidal forces responsible for emulsion stability (Bergenstahl and Claesson, 1997; McClements, 1998). Proteins tend to stabilize droplets against flocculation by a combination of electrostatic and steric repulsion, and their effectiveness is particularly sensitive to pH and ionic strength. On the other hand, nonionic surfactants stabilize emulsions principally through steric repulsion, and hence their effectiveness is much less sensitive to pH and ionic strength. The dependence of the physicochemical properties of an emulsion on environmental and processing conditions is therefore strongly influenced by the nature of the emulsifier present at the droplet surface. In this study, we examine the influence of a nonionic surfactant (Tween 20) on the physicochemical properties of a whey protein-stabilized emulsion.

A nonionic surfactant may influence the physicochemical properties of a protein-stabilized emulsion in a number of different ways: (i) competitive adsorption, (ii) specific protein–surfactant interactions, and (iii) depletion flocculation (Nylander and Ericsson, 1997; McClements, 1998). Competitive adsorption is the process whereby one kind of emulsifier molecule either partly or completely displaces another type from the droplet surface (Dickinson, 1997). For a nonionic surfactant, specific interactions usually occur between the nonpolar tail of the surfactant molecule and any exposed hydrophobic patches on the protein molecule (Mackie et al., 1993; Wilde and Clark, 1993; Nylander and Ericsson, 1997). Depletion flocculation occurs when the concentration of excess surfactant micelles in the aqueous phase exceeds a critical level and is the result of the osmotic pressure that arises because of the exclusion of micelles from a narrow region surrounding the droplet (McClements, 1998). In Tween 20-stabilized emulsions, 8 wt % surfactant is required in the aqueous phase to induce depletion flocculation (McClements, 1994). Depletion flocculation will not therefore make an important contribution to the systems studied in this work because the surfactant concentrations used were much lower than this value.

Extensive research in the area of competitive adsorption has been carried out involving a wide variety of biopolymers and small molecule surfactants (Dickinson et al., 1989, 1993; Courthaudon et al., 1991a,b; Euston et al., 1995). In these studies it was found that Tween 20 was more surface active than whey proteins and that

Physicochemical Properties of Whey Protein-Stabilized Emulsions

J. Agric. Food Chem., Vol. 46, No. 10, 1998 **3937**

the major whey protein components, β-lactoglobulin and α-lactalbumin, exhibit different displacement patterns in the presence of this surfactant. Tween 20 was more effective in completely displacing β-lactoglobulin ($R > \sim6$) (Chen and Dickinson, 1998) than α-lactalbumin ($R > \sim30$) (Courthaudon et al., 1991a; Dickinson, 1997). Unfortunately, most of this work has been carried out at room temperature and neutral pH, whereas the majority of food emulsions exist at lower pH values and undergo thermal processing (Walstra, 1993). It is therefore essential to investigate the effect of pH and heating on surfactant$-$protein interactions on the properties of oil-in-water emulsions.

In this study, the effect of Tween 20 on the physicochemical properties of whey protein isolate (WPI)-stabilized emulsions is investigated as a function of pH (pH 3$-$7) and heating (78 °C for 30 min). The samples are characterized by viscosity, creaming, and light scattering measurements. The results are explained in terms of competitive adsorption, protein$-$surfactant interactions, and colloidal interactions. The ultimate objective of this study was to determine whether emulsions with improved physicochemical properties could be developed by making use of interactions between nonionic surfactants and proteins.

MATERIALS AND METHODS

Materials. Powdered WPI was supplied by Davisco International (Le Sueur, MN). The composition of WPI specified by the manufacturer was 93.7% protein, 1.54% sulfated ash, and 5.0% moisture. The major protein components were 55$-$61% β-lactoglobulin, 19$-$22% α-lactalbumin, and 6$-$8% bovine serum albumin, with the rest being mainly immunoglobulins. Soybean oil was purchased from a local supermarket and used without further purification. Potassium phosphate monobasic sodium hydroxide buffer (0.05 M, pH 7) was purchased from Fischer Scientific (Pittsburgh, PA). The rest of the chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO). Deionized and distilled water was used in the preparation of all solutions and emulsions.

Emulsion Preparation. A 1 wt % emulsifier solution was prepared by dissolving WPI in distilled water and stirring for at least 2 h to ensure complete dispersion and dissolution of the protein. Sodium azide (0.02 wt %) was added to the WPI solution as an antimicrobial agent. An oil-in-water emulsion was prepared by homogenizing 20 wt % soybean oil and 80 wt % emulsifier solution at room temperature. The oil and emulsifier solution were blended using a high-speed blender (model 33BL79, Waring Inc., New Hartford, CT) and then passed through a high-pressure valve homogenizer three times at 6500 psi (Rannie high pressure, APV-Gaulin, model Mini-Lab 8.30H, Wilmington, MA). The homogenizer was cooled during the emulsification process by passing cold water around it, and the emulsion was cooled immediately after exiting the device by immersing the collection vessel in ice water. Consequently, the temperature of the emulsion never exceeded 40 °C, which is well below the temperature at which significant protein unfolding occurs. The freshly made emulsion was stored for 30 min at room temperature before Tween 20 was added at surfactant-to-protein molar ratios (R) between 0 and 20. The emulsions containing the Tween 20 were stirred for an additional 30 min before the samples were titrated to the appropriate pH. The oil concentration ($\sim$19.7 wt %) of the emulsions was kept constant by keeping the total amount of HCl solution (0.1 or 1 N) and water added to the samples constant. For the heating experiments, 100 mM NaCl was added 30 min after the Tween 20, and the emulsions were immediately heated at 78 °C for 30 min. The emulsions were inverted every 5 min during heating to avoid surface crust formation due to water evaporation.

When calculating the R values, we assumed that the molecular weight of the WPI was the same as that of

β-lactoglobulin (MW = 18400 Da). In fact, WPI is a complex mixture of different types of proteins, and therefore its molecular weight will differ somewhat from the value used here.

Particle Size Determination. The particle size distribution of the emulsions was measured using a laser light scattering instrument (Horiba LA-900, Irvine, CA). This instrument measures the angular dependence of the intensity of light scattered from a dilute emulsion and then indicates the particle size distribution that gives the closest fit between theoretical calculations (Mie theory) and experimental measurements. A refractive index ratio of 1.08 was used in the calculations. To avoid multiple scattering effects, the emulsions had to be diluted to droplet concentrations of $\sim$0.02 wt %. Dilution was carried out using water that had been adjusted to the pH of the sample being analyzed (using 0.1 N HCl) to minimize any pH-induced changes in the structure of any flocs. Droplet size measurements are reported as volume$-$surface mean diameters, d_{32} ($=\Sigma n_i d_i^3/\Sigma n_i d_i^2$, where n_i is the number of droplets with diameter d_i).

Rheological Measurements. The rheological properties of the emulsions were measured at 30.0 °C using a shear rheometer with a concentric cylinder measurement cell (constant stress rheometer, CS-10, Bohlin Instruments, Cranbury, NJ). The diameter of the inner cylinder was 25 mm, and the internal diameter of the outer cylinder was 27.5 mm. Emulsions were placed in the temperature-controlled (30 °C) measurement cell and allowed to equilibrate for 5 min prior to analysis. The shear rate (σ) was then recorded as the shear stress (τ) was increased from 0.167 to 126.5 s^{-1}, keeping the rate constant for 10 s at each shear rate before a measurement was made. The apparent viscosities (η_{30}) of a number of the surfactant$-$protein systems are reported at a constant shear rate of 30 s^{-1}.

Creaming Index. Ten grams of emulsion was transferred into a test tube (i.d. = 16 mm, height = 160 mm) and then stored for 24 h at 30 °C. The total height of the emulsions (H_E) and the height of the droplet-depleted lower layer (H_S) were measured. The extent of creaming was characterized by a *creaming index*: % creaming = 100 $\times$ (H_S/H_E). The creaming index provides indirect information about the extent of droplet flocculation in an emulsion: the more flocculation, the larger the particles and the faster the creaming rate (provided a three-dimensional network of aggregated particles does not form).

RESULTS AND DISCUSSION

Effect of pH on the Properties of Protein-Stabilized Emulsions. The physicochemical properties of an emulsion are influenced by the nature of the emulsifier and its interactions at the oil$-$water interface (Agboola and Dalgleish, 1996; Dalgleish and Hollocou, 1997; Casanova and Dickinson, 1998; Demetriades and McClements, 1998a$-$c). WPI-stabilized emulsions owe their stability to a combination of steric and electrostatic repulsive forces, the latter being particularly sensitive to pH and ionic strength. At pH values away from the isoelectric point (pI) of the protein, the droplet surfaces are electrically charged, being negatively charged above the pI and positively charged below it. At pH values sufficiently above or below the pI, the emulsions are stable to flocculation due to a relatively strong electrostatic repulsion between the droplets (Hunt and Dalgleish, 1994; Demetriades et al., 1997a,b; McClements, 1998). Around the pI, the electrostatic repulsive forces are reduced, leading to droplet flocculation, primarily due to the dominance of attractive van der Waals forces. This accounts for the observed increase in particle size observed around pH 4$-$6 using the light scattering technique (Figure 1). The increase in floc size is accompanied by an increase in the apparent viscosity

3938 *J. Agric. Food Chem.,* Vol. 46, No. 10, 1998

Demetriades and McClements
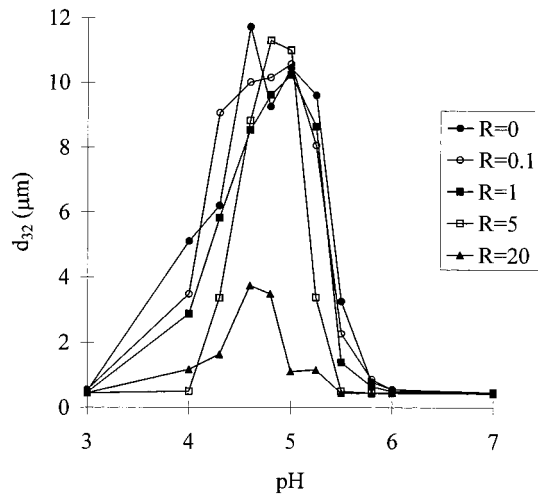
Figure 1. Influence of pH and surfactant-to-protein ratio (R) on mean droplet size (d_{32}) in 20 wt % soybean oil-in-water emulsions stabilized by WPI in the presence of Tween 20.
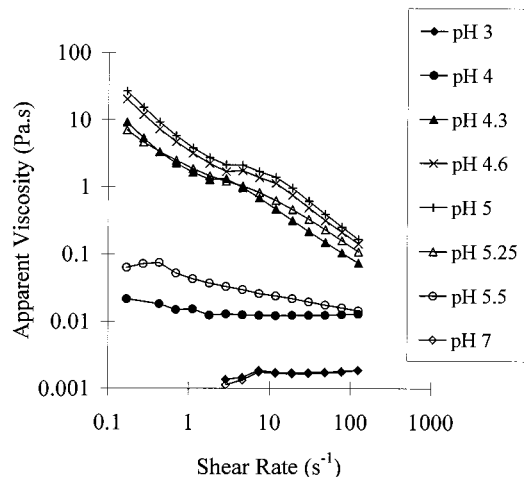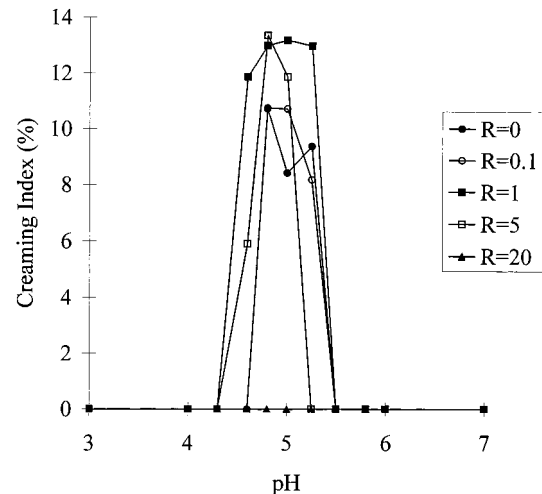

Figure 2. Influence of pH and surfactant-to-protein ratio (R) on apparent viscosity in 20 wt % soybean oil-in-water emulsions stabilized by WPI in the presence of Tween 20.
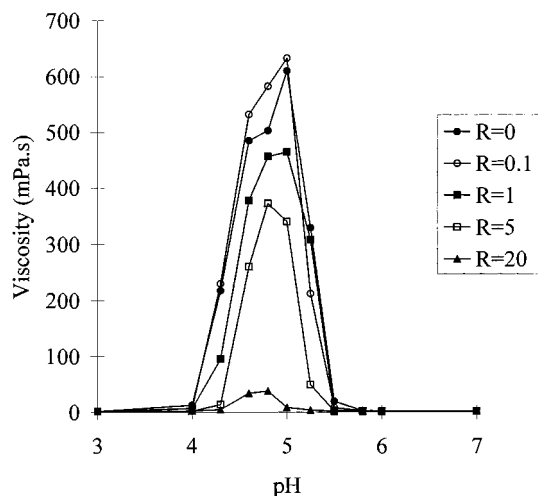
Figure 3. Influence of pH and surfactant-to-protein ratio (R) on apparent viscosity (η_{30}) in 20 wt % soybean oil-in-water emulsions stabilized by WPI in the presence of Tween 20.

of the emulsion (Figures 2 and 3), pronounced shear-thinning behavior (Figure 2), and enhanced creaming (Figure 4). The increase in viscosity occurs because the flocs offer greater resistance to flow than the individual
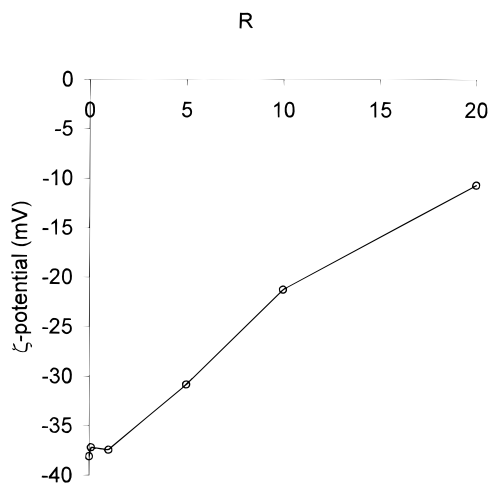

Figure 4. Influence of pH and surfactant-to-protein ratio (R) on droplet creaming in 20 wt % soybean oil-in-water emulsions stabilized by WPI in the presence of Tween 20.

droplets when an external force is applied to the system (McClements, 1998). The shear-thinning behavior in the aggregated emulsions is due to changes in floc size and shape induced by the shearing action (Uriev, 1994). This should be contrasted to the Newtonian behavior of the nonflocculated emulsions, that is, pH 3 and 7 (Figure 2). The viscosity is reported only at shear rates above 2 s^{-1} for these emulsions because the rheometer was not sensitive enough to accurately measure their viscosities at lower shear rates.

Effect of pH on the Properties of Protein-Stabilized Emulsions Containing Tween 20. The presence of Tween 20 altered the pH dependence of the particle size distribution, creaming, and rheology of the emulsions (Figures 1, 3, and 4). For low surfactant concentrations ($R \leq 0.1$) this effect was small, but for higher concentrations ($R > 1$) there was a decrease in the maximum amount of droplet flocculation near the pI and a decrease in the width of the pH instability range as the Tween 20 concentration was increased. There are at least two possible physical phenomena that could account for these observations: (i) the surfactant could displace the protein from the droplet interface; (ii) the surfactant could form a complex with the protein. Both of these phenomena could change the colloidal interactions between the droplets in a manner that would reduce the likelihood of droplet flocculation occurring.

Donnelly (1998) recently studied the influence of Tween 20 on the displacement of WPI from the surface of emulsion droplets. She found that an increasing amount of protein displacement occurred as the Tween 20 concentration was increased, being about 14% at R = 1, 43% at $R = 5$, 61% at $R = 10$, and 64% at $R = 20$. It was not until $R = 40$ that all of the protein was completely displaced from the interface. These findings are supported by our measurements of the influence of Tween 20 on the ζ-potential of WPI-stabilized emulsions (Figure 5). The ζ-potential is a measure of the sign and magnitude of the electrical charge on the surface of the droplets (Chen and Dickinson, 1995b; Stachurski and Michalek, 1996). The ζ-potential became increasingly less negative with increasing Tween 20, changing from −38.1 to −10.7 mV as R was changed from 0 to 20 (pH 7 and 0.05 M potassium phosphate monobasic sodium hydroxide). These results indicate that the amount of

Physicochemical Properties of Whey Protein-Stabilized Emulsions

J. Agric. Food Chem., Vol. 46, No. 10, 1998 **3939**

Figure 5. Influence of surfactant-to-protein ratio (R) on ζ-potential of droplets in 20 wt % soybean oil-in-water emulsions stabilized by WPI in the presence of Tween 20 (pH 7, 0.05 M potassium phosphate monobasic sodium hydroxide).

Figure 6. Theoretical calculations on the total interaction potential in WPI and Tween 20-stabilized emulsions at different surface charges. Changes in surface charge would occur through changes in pH.

negatively charged protein at the interface decreased as the Tween 20 concentration increased. These also indicate that there was still some protein remaining at the droplet interface at $R = 20$ because the droplets still had a significant negative charge.

WPI contains a complex mixture of different types of proteins, with β-lactoglobulin and α-lactalbumin being the major ones. Several researchers have shown that Tween 20 is more surface active than both β-lactoglobulin and α-lactalbumin but that the later protein requires a higher surfactant concentration before it is completely displaced from an interface (de Feijter et al., 1987; Courthaudon et al., 1991a,b; Mackie et al., 1993; Chen and Dickinson, 1995a−c). Tween 20 is able to displace β-lactoglobulin at R values >6, whereas for α-lactalbumin, R should be >30. This accounts for the observation that all of the protein was not completely displaced from the droplets at the highest surfactant concentrations used in this study ($R = 20$). The presence of some residual protein at the interface at $R = 20$ is evident from the light scattering and rheology measurements, which both indicate a certain degree of flocculation around the pI (Figures 1 and 3). This is in contrast to emulsions stabilized only by Tween 20, where the particle size distribution and viscosity were completely independent of pH (data not shown).

Our results indicate that there was a shift in the aggregation maximum as the surfactant concentration was increased (Figures 1, 3, and 4). In the absence of Tween 20 ($R = 0$), the maximum occurs between pH 4.8 and 5, whereas at the highest Tween 20 concentration ($R = 20$), the maximum occurs at pH 4.6. This pH shift can be explained by considering differences in the competitive displacement behavior of α-lactalbumin and β-lactoglobulin in the presence of Tween 20. At $R = 20$, the only protein present at the interface should be α-lactalbumin. According to Kilara and Harwalkar (1996), these two proteins have different isoelectric points: 5.3 for β-lactoglobulin and 4.2−4.5 for α-lactalbumin. When β-lactoglobulin is displaced, the flocculation maximum should therefore shift toward the pI of the protein left at the interface, that is, α-lactalbumin. Selective protein displacement therefore alters the bulk physicochemical properties of the emulsion.

Numerical calculations of the influence of emulsifier characteristics on the colloidal interactions between
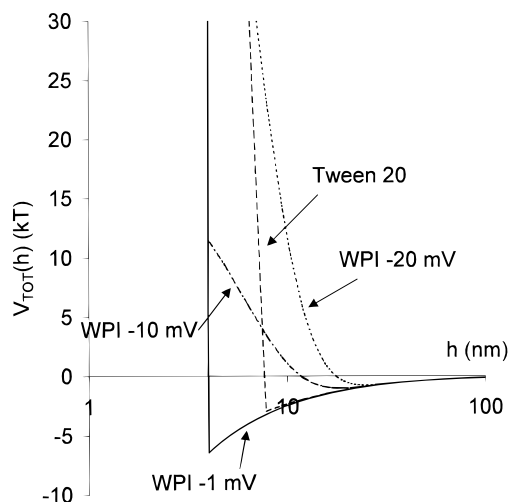
emulsion droplets are shown in Figure 6. The curves were calculated by assuming that the overall interaction potential was the sum of steric, electrostatic, and van der Waals interactions:

$$V_{TOT}(h) = V_{steric}(h) + V_{elec}(h) + V_{VDW}(h) \qquad (1)$$

$V_{TOT}(h)$, $V_{steric}(h)$, $V_{elec}(h)$, and $V_{VDW}(h)$ are the total, steric, electrostatic, and van der Waals interaction potentials, and h is the surface-to-surface droplet separation (Hunter, 1986; Israelachvilli, 1992; McClements and Dungan, 1997). The parameters used in the calculations are listed in Demetriades and McClements (1998a). Droplets tend to aggregate when there is a deep minimum in the interaction potential.

The calculations indicate that Tween 20-stabilized emulsions have a shallow minimum in the interaction potential at a separation of ~9 nm (Figure 6) and would therefore be expected to form weak flocs. In practice, Tween 20-stabilized emulsions do not flocculate, which suggests that some other factors must also be important, for example, reduction of the van der Waals attraction due to the presence of the interfacial membrane (Israelachvili, 1992). Nevertheless, the calculations do show that the steep increase in the repulsion between the droplets due to steric effects occurs at a distance that is further out from the droplet surface for Tween 20 than for WPI. In the absence of Tween 20, WPI emulsions would only be stable against aggregation if there were a large electrical charge on the droplet surfaces (i.e., ζ below −10 mV). For this reason, they tend to aggregate when the pH moves toward the pI of the proteins and the droplets lose their charge. The displacement of some of the protein from a droplet interface by Tween 20 would be expected to improve an emulsions stability against aggregation near the protein pI because its predominant mode of action is steric repulsion, which is relatively insensitive to pH (Hunter, 1986).

Our results suggest that the increased stability of emulsions to flocculation in the presence of relatively high concentrations of Tween 20 is principally due to the displacement of protein from the interface by nonionic surfactant. An alternative explanation is that the Tween 20 formed a complex with the whey proteins

3940 *J. Agric. Food Chem.,* Vol. 46, No. 10, 1998

Demetriades and McClements
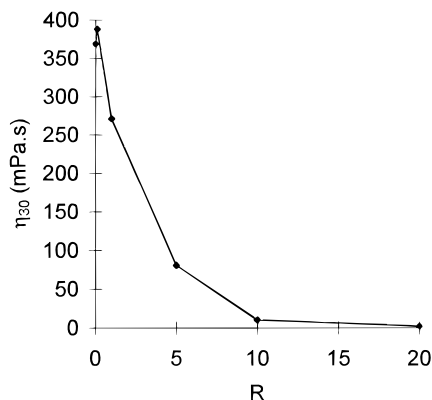
Figure 7. Influence of heating (78 °C for 30 min) and surfactant-to-protein ratio (R) on apparent viscosity (η_{30}) in 20 wt % soybean oil-in-water emulsions stabilized by WPI in the presence of Tween 20. [NaCl] = 100 mM.

Figure 8. Influence of heating (78 °C for 30 min) and surfactant-to-protein ratio (R) on droplet size (d_{32}) in 20 wt % soybean oil-in-water emulsions stabilized by WPI in the presence of Tween 20. [NaCl] = 100 mM.

at the droplet interface, which reduces the likelihood of droplet aggregation. It is known that Tween 20 forms 1:1 complexes with β-lactoglobulin at interfaces (Wilde and Clarke, 1993); however, we did not observe any significant change in emulsion properties for $R = 1$. On the contrary, the major change in emulsion properties occurred when $R > 5$, which closely corresponds to the surfactant concentration at which major protein displacement occurs (Figure 5) (Donnelly, 1998). This strongly supports the suggestion that the most likely influence of Tween 20 on the emulsion properties is through protein displacement.

Effect of Tween 20 on Heating Profile of Emulsions. In a previous study, it was shown that there was extensive droplet flocculation in WPI-stabilized emulsions at pH 7 when they were heated between 75 and 80 °C and that this caused an increase in apparent viscosity and a decrease in creaming stability (Demetriades et al., 1997b). The change in emulsion physicochemical properties was salt dependent, with the maximum effect occurring at 100 mM NaCl—the highest salt concentration used in the experiment. During that study, it was concluded that droplet flocculation in the heat-treated samples was due to thermally induced protein unfolding followed by aggregation through hydrophobic interactions and thiol−disulfide interchanges. The salt enhanced these interactions because it shielded the electrostatic repulsion between the droplets, thus allowing them to get closer together.

In the present study, WPI-stabilized emulsions containing Tween 20 ($R = 0-20$) and 100 mM NaCl were heated at 78 °C for 30 min. The emulsion viscosity (η_{30}) and average droplet diameter (d_{32}) showed a decrease in droplet aggregation with increasing Tween 20 concentration (Figures 7 and 8). At $R = 20$ there was little droplet aggregation upon heating, which is probably because the β-lactoglobulin is completely displaced from the droplet interface by Tween 20 (Chen and Dickinson, 1995b). Experiments with emulsions stabilized only by Tween 20 indicated that there was no change in the particle size distribution, rheology, or creaming stability after they were heated under the same conditions (data not shown).

β-Lactoglobulin has a free sulfhydryl group that is capable of forming intermolecular disulfide bonds with its neighbors on the same or different droplets, whereas α-lactalbumin does not (Dickinson and Matsumura, 1991; McClements et al., 1993; Monahan et al., 1993, 1996; Stevenson et al., 1996; Damodaran and Anand,
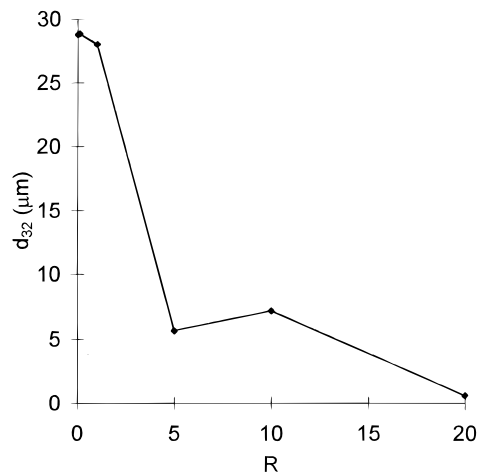
1997). One of the reasons for the observed decrease in flocculation with increasing R may therefore be the preferential displacement of the β-lactoglobulin. At $R = 10$ there was still some aggregation, even though it was assumed that this molar ratio was above the concentration required to fully displace β-lactoglobulin from the interface. This deviation can be explained by considering protein−protein interactions in solution. A number of researchers have shown that in the absence of other proteins, α-lactalbumin denaturation is reversible, whereas in the presence of other proteins, such as β-lactoglobulin, it is irreversible (Paulsson and Visser, 1992; Corredig and Dalgleish, 1995; Stevenson et al., 1996). The free sulfhydryl group of β-lactoglobulin acts as a reducing agent on α-lactalbumin disulfide bonds, therefore inducing thiol−disulfide interchanges. At $R = 10$, the two proteins can interact, thus giving rise to disulfide interactions. At $R = 20$, though, the interfacial load of α-lactalbumin is substantially reduced, and the difference is made up of Tween 20 molecules.

On the basis of the above results, it seems that the additional steric repulsion caused by the incorporation of Tween 20 molecules at the interface is capable of preventing droplets from aggregating during heating, provided the interfacial surfactant concentration is sufficiently high.

Conclusion. This study has shown that Tween 20 modifies the physicochemical properties of WPI-stabilized emulsions. At relatively high nonionic surfactant concentrations ($R = 20$), the majority of the whey proteins are displaced from the surface of the droplets, which reduces the sensitivity of the emulsion properties to pH. At moderate surfactant concentrations ($R = 5-20$), most of the β-lactoglobulin would be expected to be displaced from the surface, but the α-lactalbumin should remain. As a consequence, there is a narrowing of the pH-instability range of the emulsions and a shift of the maximum instability to lower pH values (i.e., closer to the pI of α-lactalbumin). In addition, the stability of the emulsion to flocculation upon heating is increased because α-lactalbumin-stabilized droplets are less prone to heat-induced flocculation than β-lactoglobulin-stabilized droplets. Low concentrations of surfactant ($R \leq 1$) had little impact on the stability of the emulsions, although they could cause an appreciable decrease in emulsion viscosity at the protein pI. A

Physicochemical Properties of Whey Protein-Stabilized Emulsions

J. Agric. Food Chem., Vol. 46, No. 10, 1998 **3941**

better understanding of the role that ingredient interactions and processing conditions play in determining emulsion properties will enable food manufacturers to develop products with improved physicochemical properties. By using a combination of WPI and Tween 20, it is possible to produce emulsions with textural and stability characteristics that differ significantly from emulsions stabilized by only one of these emulsifiers. Thus, it is possible to produce emulsions with specific physicochemical properties by making use of interactions between surfactants and proteins.

LITERATURE CITED

Agboola, S. O.; Dalgleish, D. G. Kinetics of the calcium-induced instability of oil-in-water emulsions: studies under quiescent and shearing conditions. *Lebensm.-Wiss. -Technol.* **1996**, *29*, 425–432.

Bergenstahl, B. A.; Claesson, P. M. Surface forces in emulsions. In *Food Emulsions*, 3rd ed.; Friberg, S. E., Larsson, K., Eds.; Dekker: New York, 1997; pp 57–109.

Casanova, H.; Dickinson, E. Influence of protein interfacial composition on salt stability of mixed casein emulsions. *J. Agric. Food Chem.* **1998**, *46*, 72–76.

Chen, J.; Dickinson, E. Protein/surfactant interfacial interactions Part 1. Flocculation of emulsions containing mixed protein + surfactant. *Colloids Surf. A* **1995a**, *100*, 255–265.

Chen, J.; Dickinson, E. Protein/surfactant interfacial interactions Part 2. Electrophoretic mobility of mixed protein + surfactant. *Colloids Surf. A* **1995b**, *100*, 267–277.

Chen, J.; Dickinson, E. Protein/surfactant interfacial interactions Part 3. Competitive adsorption of protein + surfactant in emulsion. *Colloids Surf. A* **1995c**, *101*, 77–85.

Chen, J.; Dickinson, E. Viscoelastic properties of protein-stabilized emulsions: effect of protein-surfactant interactions. *J. Agric. Food Chem.* **1998**, *46*, 91–97.

Corredig, M.; Dalgleish, D. G. A differential microcalorimetric study of whey proteins and their behavior in oil-in-water emulsions. *Colloids Surf. B* **1995**, *4*, 411–422.

Cosgrove, T.; White, S. J.; Zarbakhsh, A.; Heenan, R. K.; Howe, A. M. Small-angle scattering studies of sodium dodecyl sulfate interactions with gelatin. 1. *Langmuir* **1995**, *11*, 744–749.

Courthaudon, J.-L.; Dickinson, E.; Matsumura, Y.; Clark, D. C. Competitive adsorption of β-lactoglobulin + Tween 20 at the oil–water interface. *Colloids Surf.* **1991a**, *56*, 293–300.

Courthaudon, J.-L.; Dickinson, E.; Matsumura, Y.; Williams, A. Influence of emulsifier on the competitive adsorption of whey proteins in emulsions. *Food Struct.* **1991b**, *10*, 109.

Dalgleish, D. G.; Hollocou, A. L. Stabilization of protein-based emulsions by means of interacting polysaccharides. In *Food Colloids, Proteins, Lipids and Polysaccharides*; Dickinson, E., Bergenstahl, B., Eds.; The Royal Society of Chemistry: Cambridge, U.K., 1997; p 236.

Damodaran, S.; Anand, K. Sulfhydryl-disulfide interchange-induced interparticle protein polymerization in whey protein-stabilized emulsions and its relation to emulsion stability. *J. Agric. Food Chem.* **1997**, *45*, 3813–3820.

Das, K. P.; Kinsella, J. E. pH dependent emulsifying properties of β-lactoglobulin. *J. Dispos. Sci. Technol.* **1989**, *10* (1), 77–102.

de Feijter, J. A.; Benjamins, J.; Tamboer, M. Adsorption displacement of proteins by surfactants in oil-in-water emulsions. *Colloids Surf.* **1987**, *27*, 243–266.

Demetriades, K.; McClements, D. J. Influence of dextran sulfate and electrolyte on the flocculation of oil-in-water emulsions stabilized by a nonionic surfactant. *J. Agric. Food Chem.* **1998a**, *46*, 3929–3935.

Demetriades, K.; McClements, D. J. Influence of dextran sulfate and electrolyte on the flocculation of oil-in-water emulsions stabilized by whey protein *J. Food Sci.* **1998b**, submitted for publication.

Demetriades, K.; McClements, D. J. Influence of sodium dodecyl sulfate on the physicochemical properties of whey protein-stabilized emulsions. Submitted to *Colloids Surf. A* **1998c**.

Demetriades, K.; Coupland, J. N.; McClements, D. J. Physical properties of whey protein stabilized emulsions as related to pH and ionic strength. *J. Food Sci.* **1997a**, *62* (2), 342–347.

Demetriades, K.; Coupland, J. N.; McClements, D. J. Physicochemical properties of whey protein stabilized emulsions as affected by heating and ionic strength. *J. Food Sci.* **1997b**, *62* (3), 462–467.

Dickinson, E. Properties of emulsions stabilized with milk proteins: overview of some recent developments. *J. Dairy Sci.* **1997**, *80*, 2607–2619.

Dickinson, E.; Matsumura, Y. Time-dependent polymerization of β-lactoglobulin through disulfide bonds at the oil–water interface in emulsions. *Int. J. Biol. Macromol.* **1991**, *13*, 26–30.

Dickinson, E.; Rolfe, S. E.; Dalgleish, Competitive adsorption in oil-in-water emulsions containing α-lactalbumin and β-lactoglobulin. *Food Hydrocolloids* **1989**, *3*, 193.

Dickinson, E.; Owusu, R. K.; Tan, S.; Williams, A. Oil-soluble surfactants have little effect on competitive adsorption of α-lactalbumin and β-lactoglobulin in emulsions. *J. Food Sci.* **1993**, *58* (2), 295–298.

Donnelly, J. L. Tween 20 and Whey Protein Isolate Effects on the Oxidative Stability of Mehaden Fish Oil Emulsions. M.S. Thesis, Department of Food Science, University of Massachusetts, Amherst, 1998.

Euston, S. E.; Singh, H.; Munro, P. A.; Dalgleish, D. G. Competitive adsorption between sodium caseinate and oil-soluble and water-soluble surfactants in oil-in-water emulsions. *J. Food Sci.* **1995**, *60*, 1124.

Gelin, J. L.; Poyen, L.; Courthaudon, J. L.; Le Meste, M.; Lorient, D. Structural changes in oil-in-water emulsions during the manufacture of ice-cream. *Food Hydrocolloids* **1994**, *8*, 299.

Goff, H. D.; Jordam, W. K. Action of emulsifiers in promoting fat destabilization during the manufacture of ice-cream. *J. Dairy Sci.* **1989**, *72*, 18.

Harrison, I. M.; Meadows, J.; Robb, I. D.; Williams, P. A. Competitive adsorption of polymers and surfactants at the solid/liquid interface. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 3919–3923.

Hunt, J. E.; Dalgleish, D. G. Effect of pH on the stability and surface composition of emulsions made with whey protein isolate. *J. Agric. Food Chem.* **1994**, *42*, 2131–2135.

Hunt, J. E.; Dalgleish, D. G. The effect of the presence of KCl on the adsorption behaviour of whey protein and caseinate in oil-in-water emulsions. *Food Hydrocolloids* **1996**, *10* (2), 159–165.

Hunter, R. J. *Foundations of Colloid Science*; Oxford University Press: Oxford, U.K., 1986; Vol. 1.

Israelachvilli, J. N. *Intermolecular and Surface Forces*, 2nd ed.; Academic Press: San Diego, CA, 1992.

Kilara, A.; Harwalkar, V. R. Denaturation. In *Food Proteins and Characterization*; Nakai, S., Modler, H. W., Eds.; VCH Publishers: New York, 1996.

Lucassen-Reynders, E. H. Competitive adsorption of emulsifiers 1. Theory for adsorption of small and large molecules. *Colloids Surf. A* **1994**, *91*, 79–88.

Mackie, A. R.; Wilde, P. J.; Wilson, D. R.; Clark, D. C. Competitive effects in the adsorbed layer of oil-in-water emulsions stabilized by β-lactoglobulin-Tween 20 mixtures. *J. Chem. Soc., Faraday Trans.* **1993**, *89* (15), 2755–2759.

McClements, D. J. Ultrasonic determination of depletion flocculation in oil-in-water emulsions containing a nonionic surfactant. *Colloids Surf. A* **1994**, *90*, 25–35.

McClements, D. J. *Food Emulsions: Principles, Practice and Techniques*; CRC Press: Boca Raton, FL, 1998.

McClements, D. J.; Dungan, S. R. Effect of interactions on the rate of interdroplet heterogeneous nucleation in oil-in-water emulsions. *J. Colloid Interface Sci.* **1997**, *186*, 17−28.

McClements, D. J.; Monahan, F. J.; Kinsella, J. E. Disulfide bond formation affects stability of whey protein isolate emulsions. *J. Food Sci.* **1993**, *58*, 1036−1039.

Monahan, F. J.; McClements, D. J.; Kinsella, J. E. Polymerization of whey proteins in whey protein stabilized emulsions. *J. Agric. Food Chem.* **1993**, *41*, 1826−1829.

Monahan, F. J.; McClements, D. J.; German, J. B. Disulfide-mediated polymerization reactions and physical properties of heated WPI-stabilized emulsions. *J. Food Sci.* **1996**, *61* (3), 504.

Nylander, T.; Ericsson, B. Interactions between proteins and polar lipids. In *Food Emulsions*, 3rd ed.; Friberg, S. E., Larsson, K., Eds.; Dekker: New York, 1997; pp 189−234.

Paulsson, M.; Visser, H. Heat induced interactions of milk proteins studied by differential scanning calorimetry. In *Protein Interactions*; Visser, H., Ed.; VCH Publishers: Weinheim, Germany, 1992.

Pelan, B. M. C.; Watts, K. M.; Campbell, I. J.; Lips, A. On the stability of aerated milk protein emulsions in the presence of small molecule surfactants. In *Food Colloids: Proteins, Lipids and Polysaccharides*; Dickinson, E., Bergenstahl, B. R., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1997.

Stachurski, J.; Michalek, M. The effect of ζ potential on the stability of a nonpolar oil-in-water emulsions. *J. Colloid Interface Sci.* **1996**, *184*, 433−436.

Stevenson, E. M.; Law, A. J. R.; Leaver, J. Heat-induced aggregation of whey proteins is enhanced by addition of thiolated β-casein. *J. Agric. Food Chem.* **1996**, *44*, 2825−2828.

Uriev, N. B. Structure, rheology and stability of concentrated disperse systems under dynamic conditions. *Colloids Surf. A* **1994**, *87*, 1−14.

Walstra, P. Introduction to aggregation phenomena in food colloids. In *Food Colloids and Polymers: Stability and Mechanical Properties*; Dickinson, E., Walstra, P., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1993.

Wilde, P. J.; Clark, D. C. The competitive displacement of β-lactoglobulin by Tween 20 from oil−water and air−water interfaces. *J. Colloid Interface Sci.* **1993**, *155*, 48−54.