

Influence of Water-Soluble Nonionic Emulsifier on the Rheology of Heat-Set Protein-Stabilized Emulsion Gels

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The influence of nonionic emulsifier Tween 20 [polyoxyethylene (20) sorbitan monolaurate] on the small-deformation shear rheological behavior of β -lactoglobulin emulsion gels has been investigated. Measurements are reported for heat-set oil-in-water emulsion gels (5–10 wt % protein, 38 wt % oil, pH 7) containing surfactant added after homogenization but prior to heat treatment (30 min at 90 °C). Storage and loss moduli (frequency 1 Hz) and protein surface coverages of emulsion gels containing 6, 7, and 8 wt % protein have been determined at 30 °C. Comparison with results from the equivalent rheological experiments on pure β -lactoglobulin systems (12–14 wt %, pH 7) shows that the incorporation of fine emulsion droplets greatly reduces the overall concentration of protein required to make a self-supporting gel. For constant oil content and thermal gelation conditions, the emulsion gel strength is very sensitive to protein content and surfactant/protein molar ratio R . In particular, the storage modulus has been shown to increase at low emulsifier contents ($R \approx 1$), to decrease at intermediate emulsifier contents ($R \approx 2$), and then at high emulsifier contents ($R \geq 4$) either to increase again or to remain low depending on the protein content. This behavior can be explained in terms of the effect of system composition on the balance between the different kinds of interfacial and bulk protein–surfactant interactions.

Keywords: *Emulsion gel; β -lactoglobulin; protein–emulsifier interaction; gel strength; Tween 20; competitive adsorption; controlled stress rheometry; thermal denaturation; whey proteins*

The gel state has been defined by physical chemists as a soft, elastic, solvent-rich solid state made from a connected assembly of macromolecules or colloidal particles (Bungenberg de Jong, 1949; Flory, 1974; Burchard and Ross-Murphy, 1990; Almdal *et al.*, 1993). Depending on whether the building blocks are cross-linked polymer molecules or aggregated colloidal particles, the network structures are conventionally classified as being either “polymer gels” or “particle gels”. Where the surface of the aggregating particles is coated with polymer, or where filler particles are incorporated into an existing macromolecular network, the resulting microstructure may have some of the characteristics of both a particle gel and a polymer gel.

Soft solid-like dairy colloids like cheese or yogurt are examples of particle gels containing milk proteins (Dickinson and Stainsby, 1982; Dickinson, 1992). The constituent aggregating protein “particles” range in size over several orders of magnitude—milk protein molecules (~ 5 nm); small whey protein aggregates or casein submicelles (~ 50 nm); large whey protein aggregates, small homogenized emulsion droplets, or casein micelles (~ 0.5 μ m); native milk fat globules or large homogenized emulsion droplets (~ 5 μ m). Depending on the type of protein(s) involved, the conversion of a liquid-like dispersion of milk protein particles into a solid-like protein gel can be triggered in a number of different ways: by lowering the pH toward the isoelectric point, by heat denaturation of the whey protein(s), by high-pressure processing, by treatment with enzymes (e.g. chymosin), or by addition of divalent counterions (e.g. calcium ions). For any particular set of processing conditions, the final gel structure and rheology are dependent on the particle concentration and on the nature and strength of the interparticle interactions

during the gelation process (Dickinson, 1994). In the present paper we are concerned with the thermal gelation of oil-in-water emulsions containing the globular whey protein β -lactoglobulin.

The functional properties of whey proteins are of continuing industrial importance and research interest (de Wit, 1989; Morr and Ha, 1993). In particular, research attention has been directed in recent years toward understanding the rheology and gelation mechanisms of whey protein mixtures (concentrates and isolates) and most especially pure β -lactoglobulin (Mulvihill and Kinsella, 1987, 1988; Shimada and Cheftel, 1988, 1989; Paulsson and Dejmek, 1990; Stading and Hermansson, 1990; Matsudomi *et al.*, 1991, 1992; Hsieh *et al.*, 1993; Huang *et al.*, 1994; Matsuura and Manning, 1994). The gel microstructure and rheology are sensitively dependent on the delicate balance of attractive and repulsive forces between the aggregating protein molecules during and after gel formation (Clark and Lee-Tuffnell, 1986; Clark and Ross-Murphy, 1987). The major whey protein β -lactoglobulin plays a predominant role in determining the thermal gelling behavior of milk proteins. Depending on the conditions, a solid self-supporting heat-set gel can be produced at neutral pH conditions by thermal denaturation of β -lactoglobulin at concentrations above ca. 8–15 wt %. The gelation onset temperature is around 80–85 °C and is dependent on pH (Paulsson and Dejmek, 1990) and the type and concentration of ions (especially Ca^{2+}) (Foegeding *et al.*, 1992). The aggregated protein gel structure is held together by a combination of hydrogen bonding, hydrophobic interactions, and disulfide bonds (Mulvihill and Kinsella, 1987; Monahan *et al.*, 1995).

The effect of the incorporation of emulsion droplets on the microstructure and rheology of heat-set whey protein gels has been investigated over the past few years by several workers (Jost *et al.*, 1986, 1989; Aguilera and Kessler, 1988, 1989; Xiong and Kinsella,

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1991; Yost and Kinsella, 1992; McClements *et al.*, 1993; Aguilera *et al.*, 1993). Since it is established (van Vliet, 1988) that the viscoelasticity of filled polymer gels is determined by the nature of the interactions between the filler particles and the polymer matrix, one might expect the rheological properties of heat-set globular protein-stabilized emulsion gels to be sensitive to the nature of lipid-protein interactions, especially those occurring at the oil-water interface. Typically, it has been found experimentally that the incorporation of whey protein-coated emulsion droplets does lead to a substantial increase in gel strength at constant overall protein content. On the other hand, when the milk protein emulsifier is replaced by a low molecular weight emulsifier, it has been observed that the gel strength tends to decrease to an extent which is dependent on the protein/oil ratio and the nature of the emulsifier (Jost *et al.*, 1989; Xiong and Kinsella, 1991; McClements *et al.*, 1993).

In this paper we explore in more detail the rheology of heat-set concentrated emulsion gels containing the protein β -lactoglobulin, the nonionic water-soluble emulsifier Tween 20 [polyoxyethylene (20) sorbitan monolaurate], and the hydrocarbon oil *n*-tetradecane. We chose this model system because we have already studied in our laboratory (i) the competitive adsorption of β -lactoglobulin plus Tween 20 in dilute oil-in-water emulsions (Courthaudon *et al.*, 1991a,b; Chen and Dickinson, 1993), (ii) the influence of Tween 20 on the surface shear viscosity of β -lactoglobulin at the hydrocarbon oil-water interface (Courthaudon *et al.*, 1991a; Chen and Dickinson, 1995), and (iii) the effect of protein heat treatment on the interfacial and emulsion stability properties (Dickinson and Hong, 1994, 1995). In the present work, concentrated oil-in-water emulsions were first prepared with the native β -lactoglobulin as sole emulsifier and afterward gelled by heating to 90 °C in the presence of various amounts of water-soluble emulsifier. The latter was added *after* homogenization to avoid complications arising from the effect of the small-molecule surfactant on the emulsion droplet-size distribution. Our objective is to attempt to correlate the observed gel properties with the known interactions of β -lactoglobulin with this nonionic surfactant (Clark *et al.*, 1993, 1994) and with the competitive adsorption behavior determined here on the same concentrated emulsion samples.

MATERIALS AND METHODS

Materials. The bovine β -lactoglobulin (lot 91H7005, purity >99 wt %), Tween 20, and *n*-tetradecane (>99 wt %) were obtained from Sigma Chemical Co. (St. Louis, MO). The molecular masses of β -lactoglobulin and Tween 20 assumed in calculating the molar ratio R were 1.84×10^4 and 1.23×10^3 g mol⁻¹, respectively. Buffer salts were analytical grade reagents. The water was double-distilled.

Emulsion Preparation. Oil-in-water emulsions (5–10 wt % protein, 38 wt % oil, 20 mM bis-tris buffer, pH 7.0) were prepared using a laboratory-scale jet homogenizer (Burgaud *et al.*, 1990) operating at a constant pressure of 300 bar. Emulsion samples were degassed with a water pump. (Preliminary experiments showed that air bubbles entrapped during homogenization can lead to irreproducibility in the rheological measurement.) The emulsion droplet-size distribution and the volume-surface average droplet diameter d_{32} were determined using a Malvern Mastersizer S2.01. Known amounts of neat Tween 20 were mixed into the freshly made emulsion samples to give required values of the surfactant/protein molar ratio R . (For large values of R , this addition does lead to a slight reduction in the overall amounts of oil and protein present in the system; for example, in the 7 wt %

protein emulsion with $R = 8$, the oil content is reduced to 36.5 wt % and the protein content to 6.72 wt %.)

Protein Displacement from the Emulsion Droplets. After gentle stirring, the emulsion samples containing Tween 20 were left for 1 h to allow competitive adsorption to proceed. Each sample was centrifuged at 20 °C and 12000g for 15 min to separate the oil droplets from the aqueous serum phase. The latter was withdrawn with a syringe and filtered with low-protein-binding filters (0.22 μ m, Millipore). The protein concentration was determined by fast protein liquid chromatography (FPLC) as described previously (Dickinson *et al.*, 1989). The protein surface concentration (mass per unit area) was inferred from the measured amount of protein in the serum phase after centrifugation, together with the known total amount of protein used to make the emulsion and the specific surface area calculated from the average droplet diameter d_{32} .

Heat-Induced Gelation and Rheology Measurement. Gelation was induced by *in situ* heating of the sample in the small concentric cylinder cup (i.d. 25 mm, o.d. 27.5 mm) of a controlled stress Bohlin CS-50 rheometer. The sample (2 mL) of protein solution or emulsion was poured carefully into the rheometer cup at 30 °C and covered with a thin layer of low-viscosity silicone oil to prevent evaporation. The gelation protocol was as follows: heat from 30 to 90 °C at a rate of 3 K min⁻¹, maintain at 90 °C for 30 min, cool to 30 °C at a rate of 1 K min⁻¹, and maintain at 30 °C for 20 min.

The viscoelastic properties of the heat-treated samples were investigated by constant-stress dynamic oscillatory rheometry at 30 °C. This technique allows the elastic and viscous shear properties to be simultaneously determined with minimal disruption of the gel structure (Ross-Murphy, 1984, 1995). Measurements of the storage and loss moduli, G' and G'' , as a function of added emulsifier were carried out in the linear viscoelastic regime at a frequency of 1 Hz and at a maximum strain of 5×10^{-3} (i.e. 0.5%). Separate experiments on some gel samples were also carried out as a function of strain (0.1–80%) and frequency (10^{-3} –2 Hz).

RESULTS AND DISCUSSION

We have studied the rheology of concentrated heat-set β -lactoglobulin emulsion gels (5–10 wt % protein, 38 wt % *n*-tetradecane, 20 mM bis-tris, pH 7.0) with oil droplets of average volume-surface diameter d_{32} lying in the range 0.50–0.56 μ m. The average droplet size was found to decrease very slightly with increasing protein content; for the three emulsion systems studied in most detail, the d_{32} values were 0.54 ± 0.01 , 0.53 ± 0.01 , and 0.52 ± 0.01 μ m for the emulsions made with 6, 7, and 8 wt % protein, respectively. Unless otherwise stated, rheological measurements were made at 30 °C, at a strain of 0.5%, and at a frequency of 1 Hz.

We first make the general observation that the presence of dispersed droplets reduces by at least a factor of 2 the amount of protein required for gelation. For instance, a pure protein system (no oil droplets) made with 14 wt % β -lactoglobulin produces a (weak) gel with storage modulus $G' = 110 \pm 10$ Pa and loss modulus $G'' = 62 \pm 5$ Pa, whereas an emulsion gel made with just 7 wt % β -lactoglobulin has a greater solid-like character as indicated by the values $G' = 190 \pm 15$ Pa and $G'' = 36 \pm 4$ Pa. This strong reinforcement of the heat-set protein gel by protein-coated oil droplets is consistent with previous studies of the thermal gelation of whey proteins (Jost *et al.*, 1986) and also soybean globulins (Matsumura *et al.*, 1993a).

On the basis of the analysis of the aqueous phase after centrifugation, we can estimate that the amount of β -lactoglobulin adsorbed at the surface of the droplets in the 7 wt % protein emulsion is just 17% of the total protein present. This implies that the protein concentration in the aqueous phase (*excluding* that residing at the oil-water interface) in the 7 wt % protein

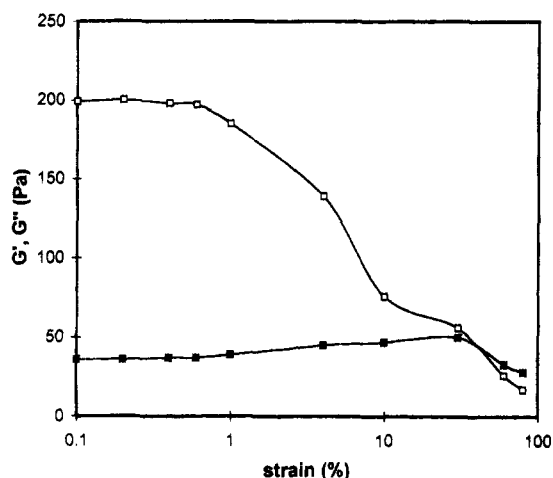


Figure 1. Strain dependence of measured rheological parameters of a heat-set emulsion gel (7 wt % β -lactoglobulin, 38 wt % n -tetradecane, 20 mM bis-tris buffer, pH 7.0, 30 °C) subjected to dynamic oscillatory deformation at a frequency of 1 Hz. The storage and loss moduli, G' and G'' , are plotted against the maximum percentage shear strain: \square , G' ; \blacksquare , G'' .

emulsion is 9.4 wt %. Such a concentration of protein, when present as a pure protein aqueous system (no oil), does not form a gel under the thermal and solution conditions employed in this study. For instance, the same heat treatment applied to 12 wt % pure β -lactoglobulin gives a liquid-like viscoelastic solution with a higher loss modulus (4.3 Pa) than storage modulus (2.8 Pa).

While the large-deformation mechanical behavior of a food gel is very relevant to its handling and eating properties (van Vliet, 1995), what we are concerned with in this study is rheological information that is indicative of structural properties, where it is essential that the rheology experiment itself does not lead to any change in structure or viscoelastic behavior. Figure 1 shows the strain dependence of the viscoelastic parameters of a heat-set emulsion gel containing 7 wt % protein measured at 30 °C and 1 Hz. We can see that the shear moduli remain constant only up to a strain of less than 1%. This narrow range of linear viscoelasticity is much more characteristic of a particle gel ("energetic gel") than a classical polymer gel ("entropic gel") (Ross-Murphy, 1984; Dickinson, 1992). All other measurements reported elsewhere refer to a maximum oscillatory strain of 0.5%; this strain gives good experimental sensitivity while still keeping the observations in the linear viscoelastic regime.

As well as investigating the final gel structure, we can use the computer-controlled Bohlin rheometer to make measurements on the emulsion systems during the heat treatment cycle. Figure 2 shows the time-dependent development of G' and G'' at 1 Hz for an emulsion containing 8 wt % protein. At time $t = 0$ and temperature $T = 30$ °C the system is liquid-like with $G' = 0.6$ Pa and $G'' = 1.2$ Pa. During the initial part of the heating period, up to around the denaturation temperature, there is a slight decrease in the complex modulus. Then, at $t \approx 17$ min and $T \approx 80$ °C, there is a sudden "crossover" of G' and G'' , indicating the formation of a gel network (Clark, 1991). This is followed by a strong increase in the storage modulus up to a relatively constant plateau value of $G' \approx 350$ Pa after about 10–20 min into the high-temperature holding period ($t \approx 35$ min, $T = 90$ °C). The cooling period is accompanied by an approximate doubling of the moduli to maximum values of $G' \approx 705$ Pa and $G'' \approx 145$ Pa after 110–120 min ($T = 30$ °C), followed by a

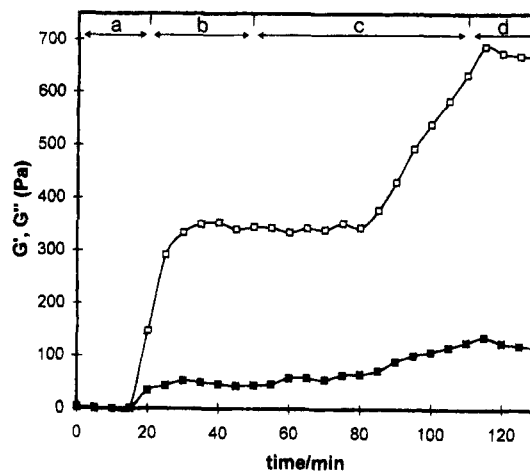


Figure 2. Development of viscoelastic parameters during thermal processing of an oil-in-water emulsion (8 wt % β -lactoglobulin, 38 wt % n -tetradecane, 20 mM bis-tris buffer, pH 7.0). The different regions of the thermal processing cycle are (a) heating from 30 to 90 °C at 3 K min⁻¹, (b) holding at 90 °C for 30 min, (c) cooling from 90 °C to 30 °C at 1 K min⁻¹, and (d) holding at 30 °C for 20 min. The storage and loss moduli, G' and G'' , at 1 Hz are plotted against the time: \square , G' ; \blacksquare , G'' .

slight fall to "final" values of $G' \approx 688$ Pa and $G'' \approx 137$ Pa at the end of the low-temperature holding period ($t = 130$ min, $T = 30$ °C).

The general form of the development of G' and G'' during the heating/cooling cycle was found to be qualitatively similar for all of the heat-set protein solutions and emulsions investigated here. The behavior is also in qualitative agreement with previous studies of the gelation of β -lactoglobulin (Paulsson and Dejmk, 1990; Huang *et al.*, 1994; Tang *et al.*, 1994) and also soybean proteins (Nagano *et al.*, 1994). Our gelation onset temperature, as judged by the location of the modulus crossover point, is slightly lower than that (84–88 °C) quoted by Paulsson and Dejmk (1990); the different heating rate could be the main reason. Recent research indicates (Monahan *et al.*, 1995; Iametti *et al.*, 1995; Hoffmann *et al.*, 1995; Renard *et al.*, 1995) that the process of aggregation and gelation of β -lactoglobulin in concentrated solutions is rather complex in molecular terms, with different kinds of protein–protein interactions involved under different solution conditions (*i.e.* pH, ionic strength) and at different processing stages (*i.e.* times, temperatures). There seems to be general agreement that electrostatic and hydrophobic interactions between adjacent hydrophobic polypeptide regions are mainly involved in the initial stages of gelation, whereas hydrogen bonding and disulfide cross-links are probably involved to a much greater extent in stabilizing the final gel structure. The pronounced increase in G' during the cooling stage in Figure 2 is in agreement with the results of Beveridge *et al.* (1984), who attributed the development of the gel strength on cooling to the formation of multiple hydrogen bonds.

When protein-coated emulsion droplets are present in a heated solution of β -lactoglobulin, the adsorbed protein molecules interact strongly with the aggregating and gelling molecules in the bulk phase. Direct microscopic evidence for the interaction between adsorbed and nonadsorbed protein has been reported (Yost and Kinsella, 1992), and it has been proposed (Aguilera *et al.*, 1993) that this interaction is mainly responsible for the high elastic modulus of concentrated whey protein emulsion gels. One obvious effect of the dispersed fat phase is to concentrate the structure-forming protein

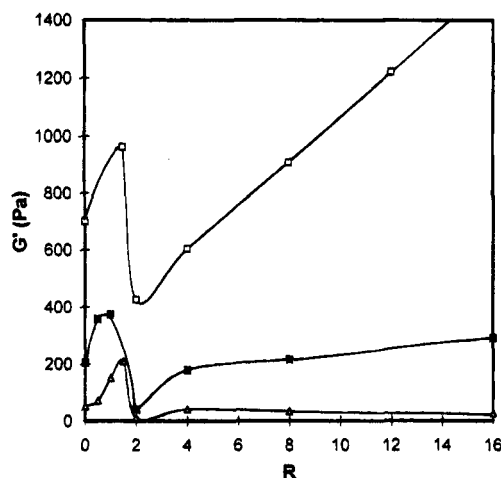


Figure 3. Influence of Tween 20 on the strength of heat-set emulsion gels (6–8 wt % β -lactoglobulin, 38 wt % oil, pH 7, 30 °C). The storage modulus G' at 1 Hz is plotted against surfactant/protein molar ratio R for three different protein concentrations: Δ , 6 wt %; \blacksquare , 7 wt %; \circ , 8 wt %.

molecules more effectively within the (aqueous phase) space available. The higher the local protein concentration, the higher is the expected gel strength due to the greater density of structurally important cross-links (Mulvihill and Kinsella, 1987). In addition, for strongly interacting adsorbed and nonadsorbed molecules, the viscoelastic protein layer around the emulsion droplets itself becomes an important load-bearing component of the total aggregated protein network structure.

The effect of Tween 20 on the elastic shear modulus of heat-set emulsion gels containing 6, 7, and 8 wt % β -lactoglobulin is shown in Figure 3. The storage modulus G' at 1 Hz (experimental reproducibility $\pm 10\%$) is plotted against the surfactant/protein molar ratio R . The first general point to be made is that the behavior is rather complex: addition of emulsifier may lead to an increase or a decrease in the gel strength depending on the total protein content and the surfactant/protein ratio. A small amount of Tween 20 (up to $R \approx 1$) produces a substantial increase in G' irrespective of the initial gel strength. Increasing the Tween 20 content to $R \approx 2$ leads to a sudden drop in G' to a value substantially below that for the surfactant-free system. Further addition of Tween 20 to $R \geq 4$ then leads to an increase again in the gel strength. The increase is rather slight for the case of the 6 wt % protein system, modest for the 7 wt % protein system, but very pronounced for the 8 wt % system. At $R = 8$, the G' value for the 7 wt % protein emulsion gel is more or less the same as that for the surfactant-free system, whereas the G' value for the 8 wt % system is about 30% larger.

We have shown previously (Dickinson *et al.*, 1993; Dickinson and Hong, 1994) that small amounts of added Tween 20 can lead to a dramatic decrease both in the surface shear viscosity of an adsorbed β -lactoglobulin layer at the oil–water interface and in the orthokinetic stability of a β -lactoglobulin-stabilized emulsion. Hence, one could reasonably expect that the small-deformation emulsion gel rheology might also be affected by surfactant-induced disruption of the protein adsorbed layer and protein displacement from the interface.

Table 1 gives the protein surface concentrations determined for the same set of liquid emulsion systems (not heat-treated) as was studied rheologically in Figure 3. We see from the data in Table 1 that at $R = 1$ there is no displacement of protein from the emulsion droplet surface. We speculate that, at this level of addition, the

Table 1. Protein Surface Concentration Γ in Freshly Made Oil-in-Water Emulsions (6–8 wt % β -Lactoglobulin, 38 wt % Oil, $d_{32} = 0.53 \pm 0.02 \mu\text{m}$, pH 7.0) Containing Various Amounts of Added Tween 20 at Surfactant/Protein Molar Ratio R

protein content (wt %)	Γ (mg m ⁻²)						
	$R = 0$	$R = 1$	$R = 2$	$R = 4$	$R = 8$	$R = 12$	$R = 16$
6	2.2	2.3	0.23	0.00	0.00		
7	1.7	1.9	1.2	0.98	0.55	0.13	0.08
8	2.1	1.8	1.4	1.2	0.70	0.19	0.19

Tween 20 is not available for disrupting the protein adsorbed layer or displacing protein from the interface because it is involved in known 1:1 complexation with β -lactoglobulin (Coke *et al.*, 1990) both at the oil–water interface and in bulk solution. Protein–surfactant complexation effects are favored in the present concentrated emulsions systems because the overall protein concentration is very high and the fraction of the protein adsorbed is relatively low ($< 20\%$). This contrasts with previous studies in this laboratory (Courthaudon *et al.*, 1991a,b; Chen and Dickinson, 1993, 1995) with systems containing either a planar interface in contact with a very dilute protein solution or a dilute emulsion of low protein emulsifier content mostly present in the adsorbed layer. While we have no convincing quantitative explanation as to why the small amounts of Tween 20 produce the observed increased storage modulus of the emulsion gel, we can speculate that the presence of complexed emulsifier leads somehow to an enhancement of the local packing density in the adsorbed layer and, therefore, of the strength and/or number of cross-links involving adsorbed protein molecules and also possibly involving aggregated protein molecules in the bulk phase.

For each of the three β -lactoglobulin contents, the data in Table 1 indicate a clear reduction in the protein surface concentration when the Tween 20 concentration is increased from $R = 1$ to $R = 2$. The magnitude of the reduction is greatest for the 6 wt % protein emulsion and lowest for the 8 wt % protein emulsion. There is a very striking correlation between these protein displacement data and the emulsion gel rheology data shown in Figure 3. What we can assume is happening is that, just above $R \approx 1$, the additional emulsifier now no longer binds strongly to the protein but rather accumulates gradually at the oil–water interface, thereby disrupting the hydrophobic protein–protein and protein–oil interactions in the adsorbed layer. This leads to a dramatic fall in adsorbed layer surface viscoelasticity and an associated increase in the rate of shear-induced coalescence (Dickinson *et al.*, 1993). With the disruption of the mechanical strength of the adsorbed protein layer and some limited displacement of protein (or protein–surfactant complex) from the emulsion droplet surface, the emulsion droplets are no longer acting as reinforcing particles within the heat-set protein gel structure, and this is reflected in a large fall in storage modulus from $R = 1$ to $R = 2$. It is interesting to note that, in both the 7 and 8 wt % protein emulsions, although increasing the Tween 20 content further from $R = 2$ to $R = 4$ produces significant extra protein displacement from the interface, there is no further decrease in the G' value. Indeed, this further addition of emulsifier now acts to increase the gel strength. Taken together with the competitive adsorption results in Table 1, this indicates that the crucial step in changing the *bulk* rheological character of the heat-set emulsion gel is probably the loss of *surface* viscoelasticity of the adsorbed layer through disruption by the surfactant of the interfacial

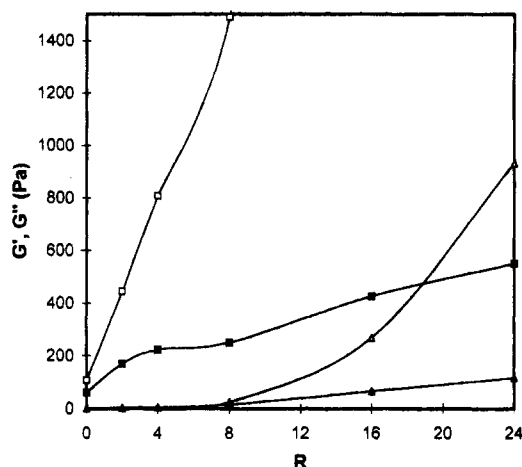


Figure 4. Influence of Tween 20 on the viscoelasticity of heat-set protein gels (12 or 14 wt % β -lactoglobulin, pH 7, 30 °C). The storage and loss moduli, G' and G'' , at 1 Hz are plotted against the surfactant/protein molar ratio R for two different protein concentrations: Δ , 12 wt %, G' ; \triangle , 12 wt %, G'' ; \square , 14 wt %, G' ; \blacksquare , 14 wt %, G'' .

protein-protein interactions (Matsumura *et al.*, 1993a,b). This disruption appears to be accompanied by some detectable, but by no means complete, displacement of adsorbed protein from the interface, in agreement with our earlier interpretation of orthokinetic emulsion stability experiments (Dickinson *et al.*, 1993).

The excluded volume effect associated with high surfactant concentrations would be expected to increase the strength of the protein-protein interactions and therefore increase the elastic modulus. Also, at concentrations well beyond its critical micelle concentration (4×10^{-3} wt %), uncomplexed and unadsorbed Tween 20 would be expected to interact extensively with the aggregated β -lactoglobulin in the bulk aqueous phase via hydrophobic interactions (Saito, 1987; Goddard and Ananthapadmanabhan, 1993) and maybe also to lead to some depletion flocculation of emulsion droplets (Dickinson, 1992). The formation of mixed micellar aggregates involving a combination of self-assembled surfactant molecules and unfolded protein molecules would tend to increase the gel strength through the generation of additional load-bearing cross-links in both β -lactoglobulin bulk gels and β -lactoglobulin emulsion gels. This explanation involving enhanced protein-protein and protein-surfactant interactions in the presence of excess Tween 20 is confirmed by the set of data in Figure 4, which shows the strong positive effect of the added emulsifier on the viscoelastic parameters of heat-set β -lactoglobulin gels (no emulsion droplets) with protein content of 12 or 14 wt %. It is noteworthy that, while the 12 wt % protein system remains liquid-like (*i.e.* $G' < G''$) in the absence of surfactant or even at modest Tween 20 concentrations ($R \leq 4$), it does form a self-supporting gel at high Tween 20 concentrations ($R > 4$). In contrast, the storage modulus of the 14 wt % protein system is a strongly increasing function of the surfactant concentration down to small values of R , and for this more concentrated protein system the presence of Tween 20 at a level of $R = 8$ (*i.e.* 7.5 wt %) leads to an increase in G' from just above 100 Pa to nearly 1500 Pa.

Returning again to consider the G' vs R data for the 8 wt % protein emulsion gel (Figure 3), we can reasonably infer that the strong increase in shear modulus over the range $2 \leq R \leq 12$ is attributable to a combination of hydrophobic complexation and excluded volume effects arising from the combined high concentrations

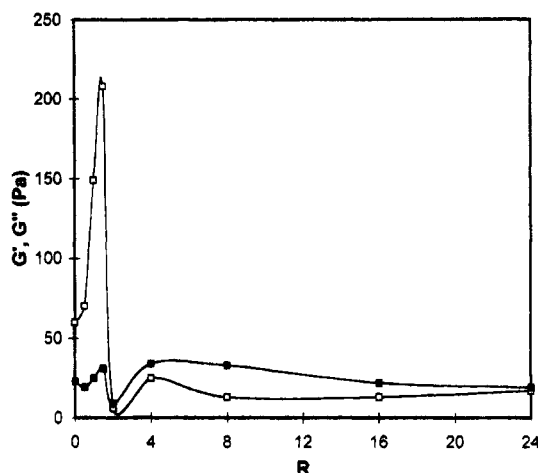


Figure 5. Influence of Tween 20 on the viscoelasticity of a heat-set emulsion gel (6 wt % β -lactoglobulin, 38 wt % oil, pH 7, 30 °C). The storage and loss moduli, G' and G'' , at 1 Hz are plotted against the surfactant/protein molar ratio R : \square , G' ; \blacksquare , G'' .

of protein and emulsifier in the bulk aqueous phase. Possibly also the high concentration of surfactant micelles leads to rheological changes associated with depletion flocculation of the emulsion droplets (Dickinson, 1992). Assuming a surface excess concentration of Tween 20 of $\Gamma = 1 \text{ mg m}^{-2}$ at the emulsion droplet surface, we can estimate an emulsion aqueous phase concentration of 6.1 wt % Tween 20 for $R = 12$. This emulsifier content in the aqueous phase is equivalent to that at $R = 6.5$ for the 14 wt % pure protein system in Figure 4. This latter system has a G' value of *ca.* 1.1 kPa, which is close to the value of $G' \approx 1.2 \text{ kPa}$ for $R = 12$ in Figure 3. These calculations indicate that the rheology of the heat-set emulsion gels at high protein content and high emulsifier concentrations can be interpreted in terms of the rheology of the equivalent mixed protein plus emulsifier aqueous system.

In contrast to the behavior at high protein content, however, the effect of an excess of emulsifier on the heat-set β -lactoglobulin emulsion gel at low protein content (6 wt %) is to inhibit gel formation. This is illustrated in Figure 5 by the plots of G' and G'' as a function of R over the range 0–24. At $R = 1$, where putative interfacial protein-surfactant complexation has its maximum influence on the bulk rheology, the system exists as a moderately strong gel with G' approximately an order of magnitude larger than G'' . However, at Tween 20 concentrations at and beyond that corresponding to substantial protein displacement (*i.e.* $R \geq 2$) (see Table 1), the loss modulus suddenly becomes predominant, indicating the existence of a liquid-like suspension rather than a gel. Even with all of the protein displaced from the emulsion droplet surface ($R \geq 4$), the protein concentration in the aqueous phase is less than 10 wt %, and this appears to be below the gelation threshold, irrespective of the amount of Tween 20 that is added to the system.

The heat-set β -lactoglobulin emulsion gels studied here can be regarded as "physical gels" since they exhibit a definite frequency dependence of G' and G'' , but without the crossover of the two moduli during the frequency sweep which is characteristic of "entanglement gels" (Clark and Ross-Murphy, 1987). Figure 6 shows a log-log plot of G' vs frequency over the range 10^{-3} –2 Hz for heat-set emulsion gels containing 5, 6.5, 8, and 10 wt % protein. With increasing protein content, the gel strength at constant frequency increases and the frequency dependence of G' decreases. At high protein

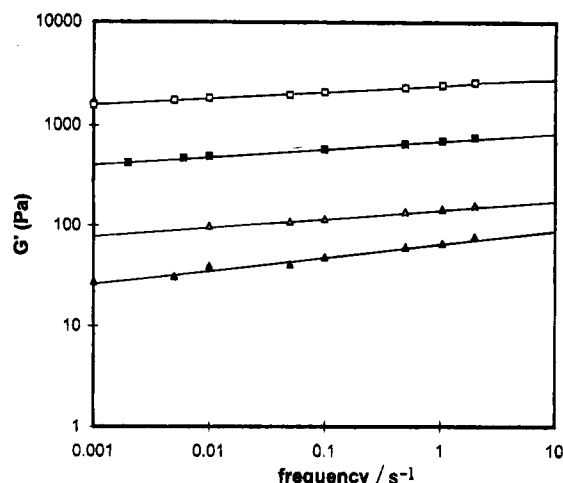


Figure 6. Frequency dependence of the storage modulus G' of heat-set emulsion gels (5–10 wt % β -lactoglobulin, 38 wt % oil, pH 7, 30 °C): Δ , 5 wt %; \triangle , 6.5 wt %; \blacksquare , 8 wt %; \square , 10 wt %.

content, therefore, the β -lactoglobulin particle gel system behaves more like a classical frequency-independent network.

CONCLUSIONS

We have shown that the shear modulus of a concentrated heat-set β -lactoglobulin emulsion gel of (approximately) constant oil volume fraction is very sensitive to the overall protein content and to the presence of different amounts of nonionic water-soluble emulsifier. While our measurements do confirm many of the broad features already reported in the literature, it can also be inferred from our results that the physicochemical processes involved are perhaps rather more complex than might have been implied by earlier interpretations, even for a system containing a relatively "simple" nonionic emulsifier such as Tween 20.

Building on earlier studies in our laboratory of the surface composition and stability of oil-in-water emulsions containing milk proteins and small-molecule surfactants, it is our aim now to try to understand how emulsion gel rheology depends on the presence of different kinds of food emulsifiers. It is quite clear from the first stage of this study that even the apparently straightforward question of whether the emulsion gel strength goes up or down upon addition of emulsifier is not simply a matter of just the chemical nature of the surfactant (nonionic, anionic, or zwitterionic). It seems that the shear modulus may increase substantially, decrease substantially, or even remain more or less the same, depending on the relative amount of emulsifier present and the overall protein content. Keeping the gelation conditions constant, we find that small additions of Tween 20 confer an increased shear modulus on a β -lactoglobulin heat-set emulsion gel, probably due to the effect of interfacial complexation. Gradually increasing amounts of Tween 20 lead to a reduced gel strength, however, presumably due to disruption of the protein adsorbed layer, followed by gradual protein displacement from the emulsion droplet surface. Further additions of emulsifier can lead either to a large increase in rheological parameters or to no significant change, depending on the protein content.

The experiments reported in this paper have been limited to a single pure protein, a single oil volume fraction, constant homogenization and thermal gelation conditions, and constant solution conditions (neutral pH, low ionic strength). The increased gel strength at low

surfactant concentrations, cautiously attributed to strong interfacial protein–emulsifier complexation, is probably specific to β -lactoglobulin and possibly even also to this particular type of nonionic surfactant. On the other hand, the decrease in gel strength due to competitive displacement of protein from the oil–water interface is likely to be a much more general effect, although the composition at which it occurs will presumably vary from one system to another, depending on the detailed nature of the protein–surfactant interactions and other factors such as pH and oil volume fraction. Much more needs to be done with other surfactants and conditions to understand how these other factors affect the physicochemical mechanisms involved.

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