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Effect of the Interfacial Layer Composition on the Properties of Emulsion Creams

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We have quantified observed differences in the microstructure and rheology of creaming emulsions stabilized by protein and low molecular weight surfactants. In this study, we made two sets of emulsions from a single parent emulsion, which differed only in their interfacial composition (i.e., either protein or surfactant). The protein studied was whey protein isolate. The ζ potential of the surfactant-stabilized emulsion was controlled by mixing anionic (SDS) and nonionic (Brij 35) surfactants to match the ζ potential of the protein-stabilized emulsion. Despite this, ultrasonic creaming measurements and confocal microscopy showed that the structures within the cream layers were different between the two sets of emulsions. The protein-stabilized emulsions appeared to slow or arrest the packing within the cream, leading to a lower density network of emulsion droplets, whereas the surfactant emulsion droplets rearranged more quickly into a well-packed, concentrated cream layer. Rheological analysis of the creams showed that despite the protein-stabilized emulsions having a lower dispersed phase volume fraction, their elastic modulus was approximately 30 times greater than that of a comparable surfactant-stabilized emulsion. These differences were caused by the ability of the protein to form a highly viscoelastic interfacial network around the droplets which may include intermolecular covalent cross-links. At close range the adhesive nature of the interaction between the layers contributes to the microstructure and rheology of concentrated emulsions. This is the first time that such well-defined emulsion systems have been studied in detail both noninvasively to look at the impact on creaming and also invasively to look at the impact on bulk rheological properties.

KEYWORDS: Mixed protein; interfacial rheology; dilatation; shear; whey; air/water interface

INTRODUCTION

Emulsions can be referred to as functional fluids in which one immiscible fluid is incorporated within another to generate structure or solubilize oil-miscible components for encapsulation and controlled release of compounds. They have applications as wide ranging as food, pharmaceuticals, oil production, agrochemicals, paints, and photography. In food systems proteins are often used to stabilize emulsions against coalescence, as they possess unique interfacial properties that can confer high levels of long-term stability (*1*). Proteins are complex, polyionic, amphiphilic macromolecules with a range of secondary and tertiary structures, and their unique interfacial properties have been studied for many years (*2*, *3*). Adsorption of proteins to an interface is governed by several factors including surface hydrophobicity and molecular flexibility. However, once adsorbed they tend to undergo rearrangements such as loss of tertiary structure and aggregation processes to form an immobile, elastic interfacial film (*2*), which is essentially formed irreversibly. These films are held together by a combination of intermolecular and intramolecular electrostatic and hydrophobic interactions, and thus, the molecular structure of the proteins can strongly influence their interfacial rheological properties (*4*, *5*). These properties have in turn often been associated with enhanced stability of emulsions and foams (*1*, *2*). In contrast, most low molecular weight surfactants form a fluid, mobile interfacial layer (*6*) which readily exchanges with the bulk surfactant pool. This marked contrast in interfacial behavior between proteins and surfactants often leads to antagonistic effects on foam and emulsion stability (*1*, *3*, *6*).

While emulsions must in general be stable to phase separation, in food systems they must also possess other important properties such as microstructure and rheology (*7*, *8*). Apart from the properties of the continuous phase, the main factors which govern emulsion rheology are the properties of the dispersed phase droplets (*8*, *9*). These include the droplet volume fraction, droplet−droplet interactions, droplet viscosity, and deformability. Considerable progress has been made in understanding the factors that influence the physicochemical properties of model emulsions. It has been shown (*10*) that there is a sharp transition from liquid- to solidlike behavior as the droplet volume fraction reaches the random packing limit, particularly

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in monodisperse systems (*11*). However, our knowledge of the structure and rheology of dispersions containing polydisperse, deformable droplets is still at an elementary stage. Emulsion droplets have a fluid internal phase and a flexible interface. The application of stress can cause circulation of the internal phase and may lead to droplet distortion (*12*). The deformation of the droplets is influenced by the viscosity of the dispersed and continuous phases and the shear field. Scaling of the viscosity of an oil-in-water emulsion with the droplet size, the viscosity of the two phases, and the interfacial tension has been shown (*9*). More recently, Mason et al. (*11*, *13*) showed that the Laplace pressure ($2\gamma/r$) and thus both the interfacial tension (γ) and the droplet radius (r) are important in relation to emulsion rheology and should be used as normalization factors. The rheology of high phase volume emulsions is also dependent on the way that the interfacial layer couples the dispersed and continuous phases. Evidence that the interfacial tension is not the only characteristic of the interface that may be a factor in the deformation of droplets has been supplied recently through the work of both Pozrikidis and Nadim. Pozrikidis studied the deformation of a liquid drop with a constant isotropic surface tension and finite surface viscosity (*14*) and found the surface viscosity acted to suppress the interfacial motion and hence reduce the magnitude of the droplet deformation. Nadim has provided results that suggest that the deformation of droplets and the measurement of the effective viscosity of the emulsion are functions of the surface shear and dilational viscosities (*15*).

It has been shown that the rheology of concentrated emulsions stabilized by proteins tends to have greater elastic moduli than that of emulsions stabilized by low molecular weight emulsifiers (*7*, *16*−*19*). Within our own laboratory, we have investigated the role of interfacial structure in the related area of the sensory perception of "creaminess" in model food emulsions (*20*). We showed that the interfacial composition of the test emulsions influenced the sensory perception of creaminess and fat content. Changes in the interfacial composition also had effects on the emulsion rheological behavior. An enhanced viscosity was found for the protein-stabilized emulsion compared to a similar emulsifier-stabilized emulsion. However, the mechanism(s) underlying this phenomenon was not entirely clear. There have been some theoretical treatments showing how an elastic interfacial layer can influence droplet deformation (*15*, *21*) and emulsion rheology (*17*), and certainly the elasticity of most globular protein interfacial layers would induce this influence. Experimental studies of the rheology of protein-stabilized emulsions have suggested that the interactions between the protein-stabilized droplets may be responsible for the enhanced rheological properties (*16*). There have been several studies looking at the interactions between interfacial protein layers on both liquid and solid supports, and although long-range interactions are dominated by electrostatic repulsion (*22*−*24*), short-range interactions can vary for different proteins (*22*). Most globular protein layers generate a large steric repulsion term (*22*, *24*), sometimes due to the formation of multilayers (*22*) or adsorbed protein aggregates (*23*), but it has also been inferred that non-DLVO hydration forces may account for experimental deviations from theory (*24*, *25*). However, the consequences of these interactions have not been fully investigated. It has also been shown that interactions between protein-stabilized emulsion droplets can influence the phase separation of the emulsion (*26*). However, as far as we are aware, there has been no systematic experimental study of the role of a protein-adsorbed layer on the microstructure and bulk rheology of the cream layer of oil-in-water (O/W) emulsions.

Table 1. Droplet and Interfacial Parameters for the Emulsion Systems Studied

	ζ potential (mV)	mean droplet diam, D_{32} (μm)	interfacial tension (mN/m)	interfacial elastic modulus, $\|E\|$ (mN/m)
WPI (pH 6)	−28.4	6.75	18.0	38.9
Brij 35	−0.4	6.75		
SDS	−79.9	6.75		
Brij 35/SDS	−25.5	6.75	8.3	1.5

The aim of this study was to investigate more thoroughly how the interfacial rheology can influence the structure and bulk rheology of O/W emulsions. Our approach was to quantify more precisely the role of the interfacial composition by creating emulsions that were as nearly identical as possible in terms of size and long-range interaction, but with varied interfacial rheological properties. This was achieved by creating an emulsion stabilized by protein (whey protein isolate) possessing an immobile, viscoelastic interface. This interface was then disrupted and displaced by the addition of surfactants (*27*, *28*) to produce a nearly identical emulsion but stabilized by surfactant. Different surfactant mixtures were used to control the surface charge and hence the ζ potential of the emulsion droplets. An investigation of the emulsion structure and rheology during creaming was undertaken.

MATERIALS AND METHODS

An oil-in-water emulsion with a dispersed phase content of 20% (w/w) was prepared consisting of *n*-tetradecane as the dispersed phase and 0.43% (w/v) whey protein isolate (Bipro, Davisco Foods International Inc., Minnesota) in a 10 mM citrate buffer at pH 6.0. The emulsion was prepared using a Waring blender using a timed shearing cycle (30 s at low speed, 30 s at rest, 2 × 30 s on high speed with 30 s of rest in between). The particle size distribution of this protein-stabilized emulsion was measured. The emulsion was then divided into two equal (90 mL) parts. To each sample was added 9.09 g of sodium citrate buffer (pH 6) ± 2.7% surfactant, and the resulting solution was stirred for 30 min. From previous measurements we know that this surfactant concentration was sufficient to displace the protein from the oil droplet surface. The data in **Table 1** show the reduction in interfacial tension induced by the surfactant was 10 mN/m. This difference has been shown in the past to be sufficient to induce displacement of protein from an interface by surfactant (*27*). The result was that two separate emulsion samples were produced with identical oil phase volumes and droplet size distributions, but one was stabilized by the original whey protein and the other by the added surfactant. The final continuous phase buffer concentration was 10 mM. The surfactants used were polyoxyethylene 23 lauryl ether (Brij 35) and sodium dodecyl sulfate (SDS) (Sigma-Aldrich, Poole, U.K.). Droplet size measurements at the end of the experiments showed no change from the initial values.

The droplet size distribution in the emulsions was measured using a Coulter LS 230 laser diffraction particle sizer (Beckman Coulter Inc., California), and the data were analyzed using an optical model for a fluid with real parts of the complex refractive index set to 1.332 and 1.391 for the continuous and dispersed phases, respectively.

The ζ potential of the emulsions was measured using a Zetasizer 3 (Malvern Instruments, United Kingdom) and calibrated using the −50 mV standards supplied by Malvern. All samples were measured having been diluted using the relevant continuous phase (separated by centrifugation). The interfacial tension between the aqueous phase of the emulsion and *n*-tetradecane was measured using the pendant drop technique.

Surface shear and bulk rheological measurements utilized a TA Instruments AR2000 controlled stress rheometer (TA Instruments Ltd., Crawley, U.K.) in controlled strain mode. In situ rheological measurements during creaming used a modified cup and bob arrangement with a cup of 150 mm depth and an inner cylinder of 40 mm height. With this arrangement we are able to monitor the bulk rheology of a sample

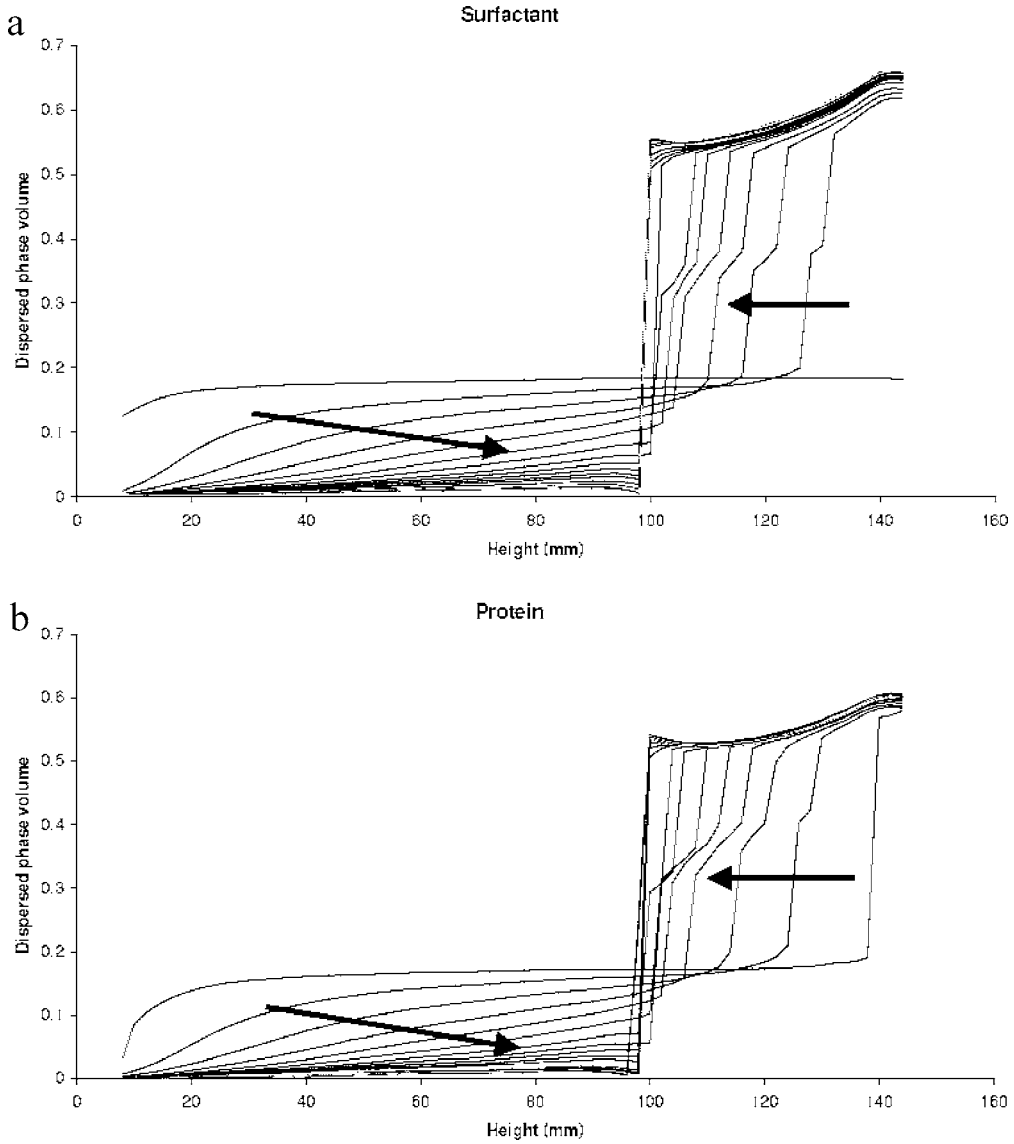

Figure 1. Dispersed phase volume as a function of height within the emulsion for WPI (**a**) and Brij 35/SDS (**b**) stabilized *n*-tetradecane-in-water emulsions. Each line represents a set of measurements taken 60 min apart over 40 h. The arrows indicate the progression with time.

at a range of heights within a creaming emulsion. The cylinder can be placed near the top of the cup to monitor the development of the creamed phase or toward the bottom of the cup to monitor the serum phase. A measuring frequency of 1 Hz and a strain of 1% were chosen as the measuring conditions, and the samples were monitored over the same time scale as the ultrasonic creaming measurements. Rheological measurements of the separated cream were carried out using a parallel-plate configuration in either steady or dynamic mode as required. Surface rheological measurements of the continuous phase at the oil/water interface were undertaken with a polished aluminum bicone (6°, 60 mm diameter) placed at the interface, over a 2 h time period again using a strain of 1% but at a frequency of 0.5 Hz. Dilatational rheological measurements were conducted using the oscillating pendant drop method (*29*) at room temperature and 0.1 Hz with a deformation of less than 4%.

Creaming profiles were assessed using measurements of ultrasonic velocity through the sample. These measurements were then related to the disperse phase volume fraction via the Urick equation (*30*). Measurements were made using an Acoustiscan system (Leeds University, United Kingdom) (*31*). Readings were taken every 2 mm over the entire height of the emulsion, to give a profile of the dispersed phase volume throughout the emulsion. Measurements continued until the majority of the oil was in the cream layer and the distribution was approaching equilibrium. All measurements were made at 20 °C.

Images of the emulsion microstructure were acquired using a Biorad 1024 confocal microscope based around a modified Nikon Optiphot microscope. Samples were placed in a specially modified cuvette which had one side replaced with a cover glass. Observations were made using a 60× oil immersion objective with a numerical aperture of 1.4. Samples were stained using Nile Red which was added to the sample in powder form and allowed to stand for several days.

RESULTS

The aim of the experiments discussed in this paper was to examine the importance of interfacial rheology in determining the bulk rheological properties of emulsion cream layers. To do this, we initially constructed two emulsions with identical size distributions (**Table 1**) and similar long-range interaction potentials but with very different interfacial rheological properties. Thus, using the method described above, two emulsions were made, one stabilized by a mixture of nonionic (Brij 35) and anionic (SDS) surfactant and the other stabilized by whey protein (WPI), both in a buffered continuous phase at pH 6.

The separation of the emulsions due to gravity was followed noninvasively using the ultrasonic technique described above. The results are shown in **Figure 1** in terms of the dispersed

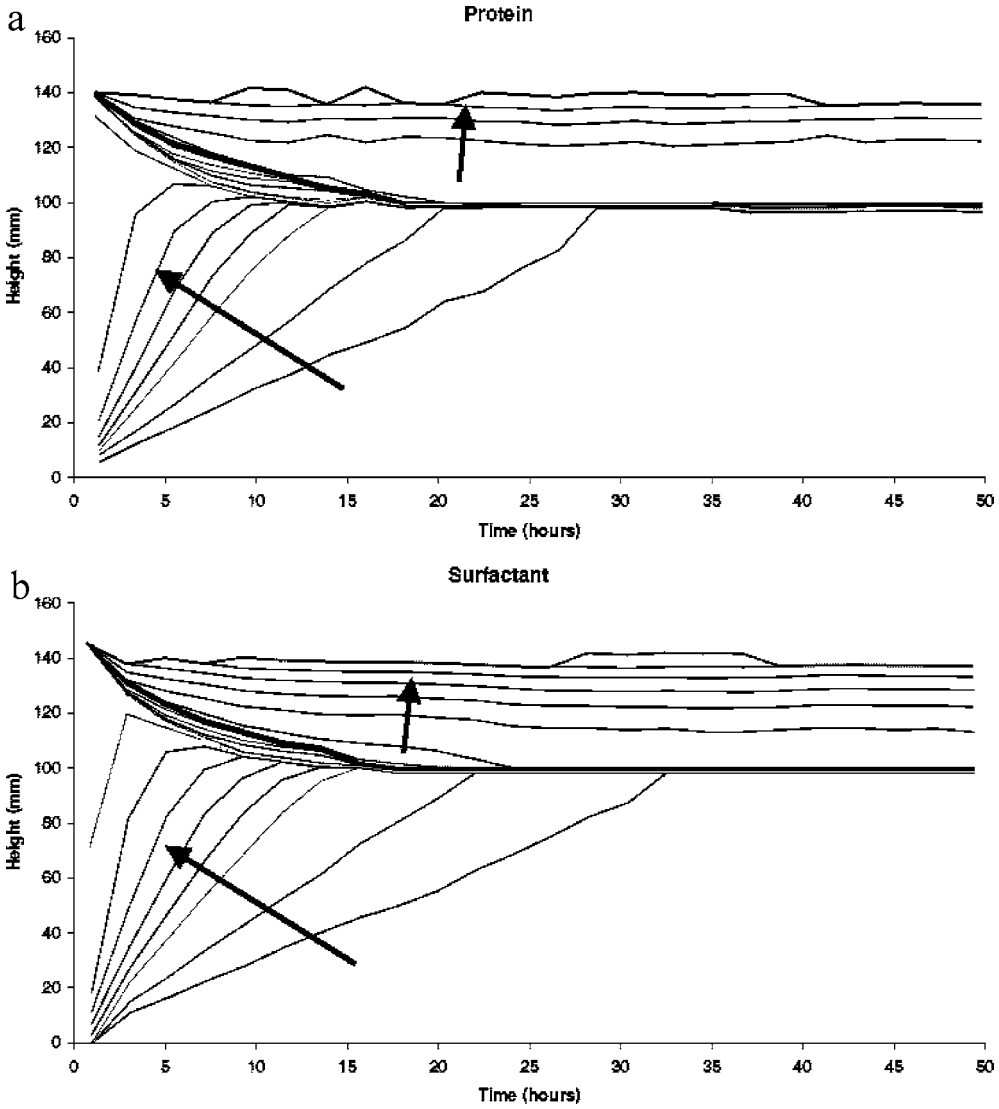

Figure 2. Dispersed phase volume contour plots of height within the emulsion as a function of time for emulsions stabilized by (**a**) protein and (**b**) surfactant. The dispersed phase volume contours are in steps of 0.02, and the arrows indicate the increasing dispersed phase volume.

phase volume as a function of height for a series of time intervals over a period of 48 h. Both emulsions show the development of a cream layer (high dispersed phase volume) at the top of the tube, which gradually increased in depth with time as the droplets migrated upward, leaving a clear serum layer at the base of the tube. The lack of a sharp boundary at the base of the creaming emulsion indicates a broad size distribution and a lack of flocculation. Because the size distributions of both emulsions were identical, the oil droplets floated upward at identical rates in both emulsions. The region that shows distinct differences is the cream layer itself. Both samples show the presence of a transient, pseudoplateau of lower density at the base of the cream which develops in the first hour or so of creaming and disappears after about 15 h in the case of the surfactant system and about 18 h in the case of the protein-stabilized system. This suggests a delay in the rearrangement into a more densely packed layer. After this 15 h/18 h period the ascending drops seem able to pack into the denser form directly. This may have been a result of the polydispersity of the emulsion leading to larger droplets ascending into the cream layer first and the packing density increasing only once the smaller droplets arrived, giving rise to a delay in rearrangement. A second difference to note between the two samples is that the final phase volume at the top of the cream layer was 0.05 greater in the surfactant-stabilized system than in the protein system, and this value was reached gradually over the period of the experiment. In the case of the protein-stabilized system the maximum phase volume at the top of the cream was reached in about 10 h.

To highlight these differences, the data from this experiment are shown in **Figure 2** in the form of contour plots of height against time. The contours represent lines of equal oil phase volume starting at 2% (lowest y values) and increasing in 2% intervals up to around 60% depending on the final phase volume at the top of the cream layer. This type of plot allows the determination of the aggregation state of the droplets as they rise toward the cream layer. The linearity of the contour lines below the cream layer shows droplets ascended with fixed velocities. This means that the droplets did not aggregate in the subcream region. These Stokes velocities can also be used to calculate a size distribution, which was found to be in good agreement with that measured by light scattering at the start of the experiment. The volume mean from the light scattering was measured as 6.2 μm, while that calculated from the Stokes velocities was 6.1 μm. The pseudoplateau at the base of the cream layer is clear in both samples and had a depth of 10 mm at its maximum extent in the protein sample but only 8 mm in depth for the surfactant-stabilized system.

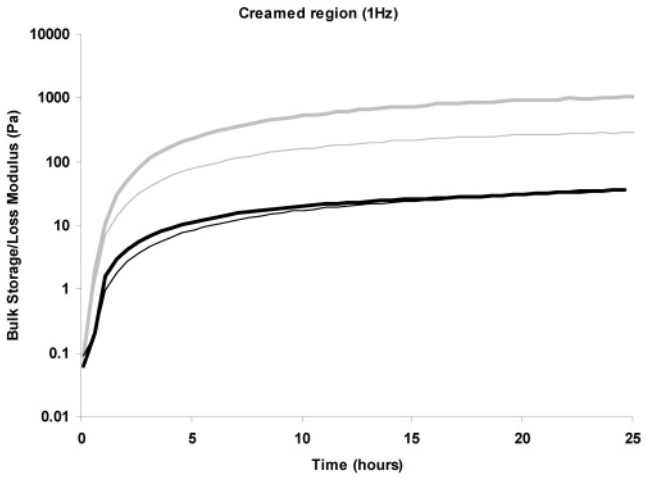

Figure 3. Storage modulus (thick line) and loss modulus (thin line) for creaming emulsions stabilized by WPI (gray) and Brij 35/SDS (black).

The interactions between emulsion droplets are at their most important in the dense cream layer and are manifested through the rheological properties of the layer. **Figure 3** shows the bulk storage and loss moduli (G' and G'') of the respective cream layers as they developed for the WPI- and Brij 35/SDS-stabilized emulsions. Measurements were made at a frequency of 1 Hz and 1% strain. These measurements were carried out on duplicates of the samples monitored in **Figures 1** and **2** and were measured over a period of 24 h. The protein-stabilized emulsion gave much higher values of the elastic modulus than the surfactant-stabilized emulsion by a factor of about 30 after 24 h and showed a large difference between the storage and loss components of the modulus. This indicates that a highly elastic system developed in comparison to the surfactant-stabilized system, which showed very similar storage and loss components. It is also clear that the moduli of both the systems were still increasing even after the cream layer had essentially fully developed (about 20 h). This indicates that the number and/or the strength of the interactions continued to increase over time even after creaming was completed. Frequency-dependent measurements undertaken in situ during the creaming of the emulsions demonstrated clearly the difference in the bulk behavior of the two systems. **Figure 4a** compares the emulsions at the start of the creaming process, demonstrating that the rheological properties of the two emulsions were identical at that stage. This is to be expected under these conditions because at this low volume fraction of 0.2, the number and strength of the interactions between emulsion droplets will not contribute significantly to the emulsion rheology. However, during creaming the behavior of the creamed layers diverges dramatically so that after 24 h of creaming (**Figure 4b**), the protein-stabilized system demonstrated clear solidlike behavior under the test conditions chosen, whereas the surfactant-stabilized system showed a high degree of flow under the same conditions. Clearly, the protein adsorbed to the oil droplets in these concentrated creams was doing more than just providing an immobile elastic coating and appears to have promoted a high degree of interaction between the droplet surfaces.

Figure 5 shows the storage (elastic) and loss (viscous) components of the modulus as a function of applied strain of the final creamed phase of each emulsion. The storage modulus showed higher values than seen in **Figure 3** for both emulsion creams indicative of the previous comment that the strength of these systems continues to increase after creaming appears

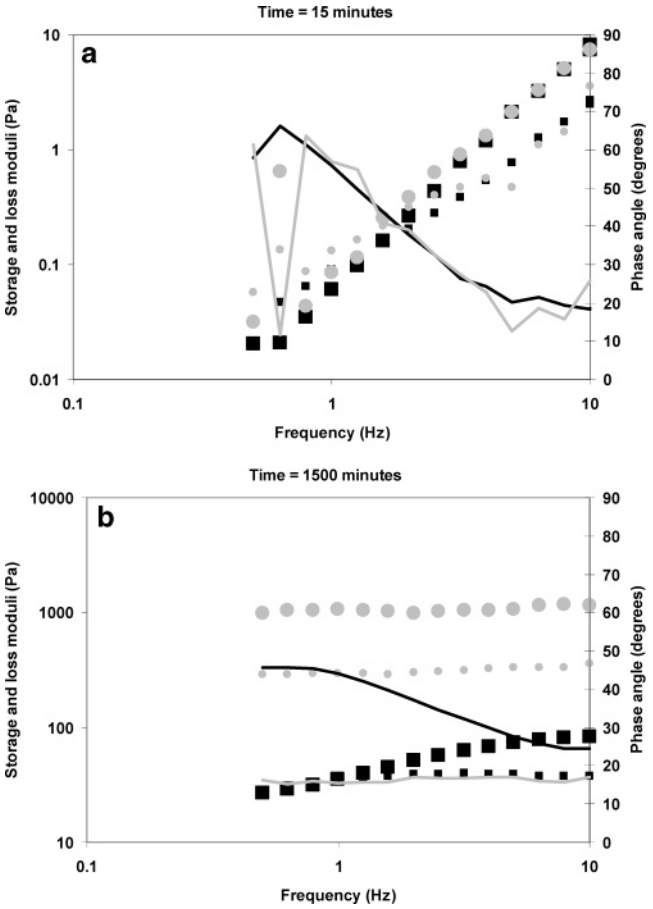

Figure 4. Storage modulus (large symbols), loss modulus (small symbols), and phase angle (lines) for WPI (gray) and Brij 35/SDS (black) stabilized emulsions plotted as a function of frequency 15 min after the start of creaming (**a**) and 1500 min after the start of creaming (**b**).

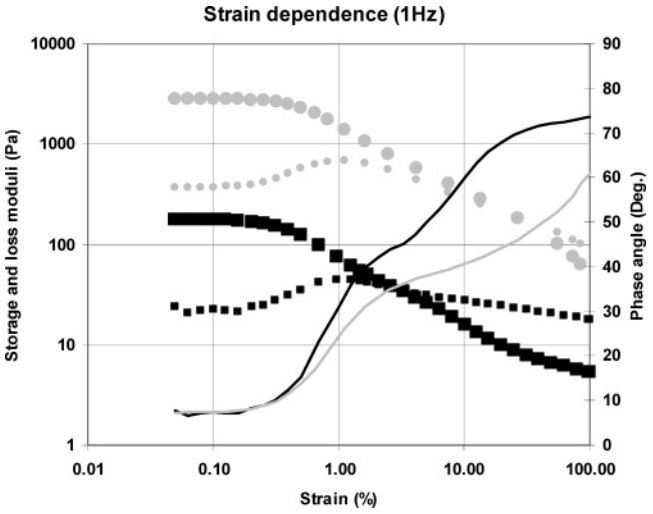

Figure 5. Storage modulus (large symbols), loss modulus (small symbols), and phase angle (lines) for WPI (gray) and Brij 35/SDS (black) stabilized emulsion cream layers plotted as a function of strain (%) at a frequency of 1 Hz.

complete. However, at 1% deformation, the modulus values were not significantly different between **Figures 3** and **5**. This was because the emulsions were demonstrating some shear thinning behavior, resulting in a decrease in the storage modulus above a "yield value" at deformations of around 0.3%. If the

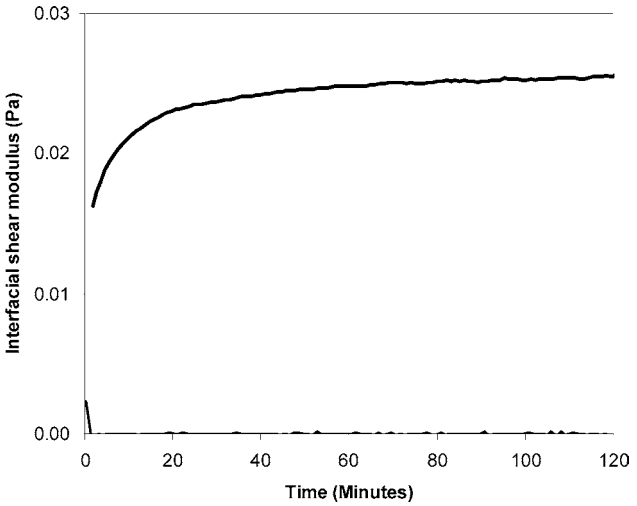

Figure 6. Interfacial shear storage modulus of the serum phase extracted from emulsions stabilized by WPI (thick line) and Brij 35/SDS (thin line) at the *n*-tetradecane/serum interface.

"bulk" rheology is due to weak interparticle interactions, then this "yielding behavior" at low deformations is to be expected. The viscous component showed a maximum in both samples at 1% strain. The nature of the sample in terms of whether it is more fluidlike or gel-like can be defined by the crossover between the storage and loss components (*32*). A phase angle of less than 45° can be considered to indicate a gel-like material. In the case of the surfactant-stabilized emulsion, the structure was broken down sufficiently to become liquidlike at a strain of 3%, whereas the protein-stabilized cream still retained a degree of gel-like behavior at strains greater than 26%, and even then the cream was at least 10 times more viscous than the surfactant-stabilized cream.

Having established that the creaming and rheological behaviors of the two systems were different, and the origin of the differences lay in the interfacial layer, it was important to look for the mechanism underlying these differences. One of the most striking properties that varied between the two systems was the interfacial rheology, particularly the shear rheology, shown in **Figure 6**. This figure and **Table 1** show the interfacial shear and dilatation storage moduli, respectively, for the protein and surfactant serum phases at the *n*-tetradecane/solution interface. The higher values for both the shear and dilatational storage moduli attained by the WPI are typical for protein-stabilized interfaces and a contributing factor to the larger bulk storage modulus in the protein-stabilized emulsions. The difference in the interfacial tension between the protein and surfactant systems will also have had an influence on the droplet deformability and is known to influence the rheology of concentrated dispersions (*9*, *11*, *13*). Measurements of the interfacial tension of the subphases against *n*-tetradecane gave values of 18.0 mN/m for the protein-stabilized system but a much lower value of 8.3 mN/m for the Brij 35/SDS system. The subsequent difference in the Laplace pressure would be expected to have an effect on the droplet deformability, leading to the protein system being more elastic at the higher volume fractions but only by about a factor of 2. Similarly, the difference in dilatational storage modulus will have restricted the deformability of protein-covered droplets, thus increasing the bulk storage modulus.

Emulsion cream layers were imaged by confocal microscopy as described above, in an attempt to explain the lower dispersed

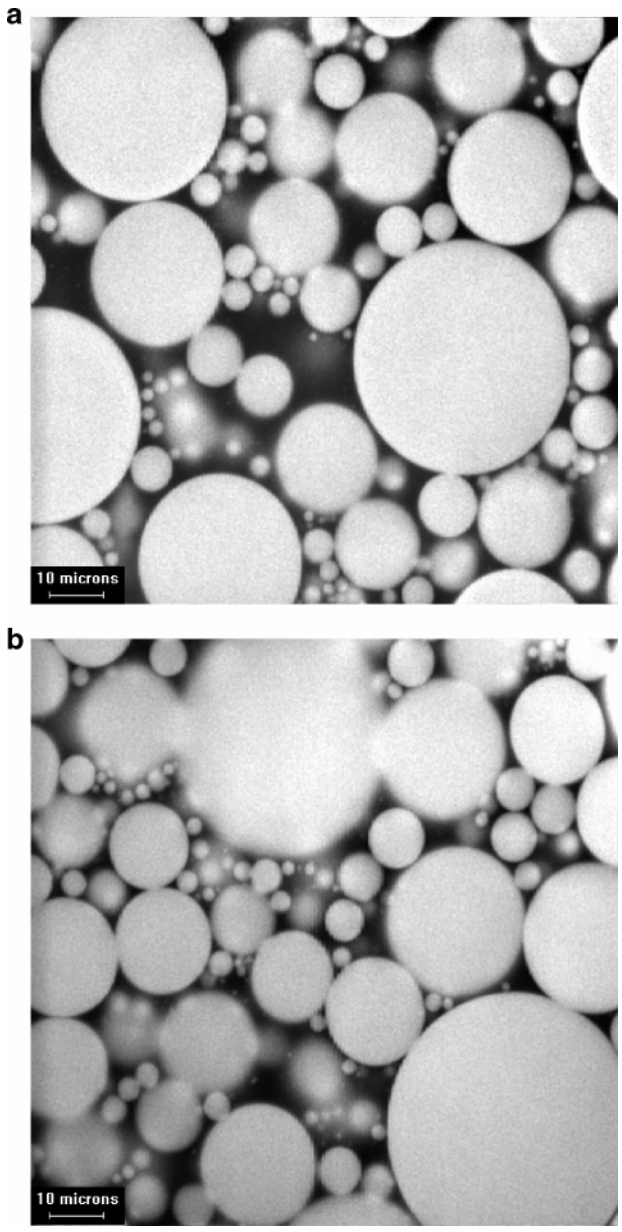

Figure 7. Confocal micrographs of the cream layer of emulsions stabilized by protein (**a**) and surfactant (**b**). Both images were taken approximately 30 μm from the container wall and are at the same magnification.

phase volume of the cream layer in protein systems shown in **Figures 1** and **2**. We surmised that there was less rearrangement of oil droplets in the protein-stabilized cream layer because of its gel-like nature, which might lead to more voids or unfilled interstitial spaces. **Figure 7** shows two images taken from near the top of the two cream layers. The image in **Figure 7a** is from the emulsion stabilized by protein and shows, as expected, a range of droplet sizes. Also visible in the image are a number of void areas that have few droplets in them. This is in contrast to the image in **Figure 7b**, which is from the top of the Brij 35/SDS-stabilized emulsion and shows fewer voids. However, one of the most telling differences is not discernible in the images. In the surfactant-stabilized systems that were investigated the voids contained small droplets that were undergoing Brownian diffusion. However, in the protein-stabilized systems there were fewer smaller drops. There were also differences in the diffusivity of the cream/serum boundary (data not shown). The full three-dimensional stacks can be seen at http://

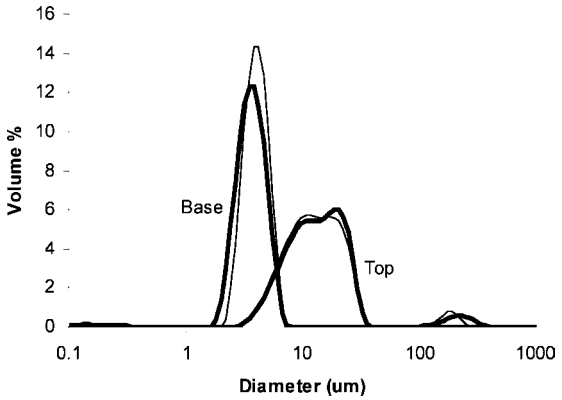

Figure 8. Size distributions of emulsion stabilized by WPI (thick lines) and Brij 35/SDS (thin lines) taken from the base and top of the cream layer after 5 days.

www.ifr.ac.uk/science/programme/F1/rheology.html and demonstrate the differences between the two systems. Aliquots taken from the base and the top of the two cream layers were measured to determine the particle size distribution. **Figure 8** shows that there were only minor differences between the samples, and the main difference was between the top and base of the cream layers, separated by about 4.5 cm. This type of segregation is to be expected as the larger droplets rise faster than the smaller ones. In this case the droplet diameters vary by about a factor of 100 between the top and the base of the cream.

DISCUSSION

The objective of the work described here was to determine the importance of the interfacial characteristics of proteins in controlling the bulk rheology of high dispersed phase volume emulsion systems. Systems were chosen to have particular interfacial properties such as a high interfacial elasticity or a specific net charge. The two samples focused on here had similar long-range interaction potentials while having very different interfacial rheological properties. The ionic strength in all the systems studied was kept constant at 10 mM. Thus, using eq 1,

$$\kappa = \left(\frac{2e^2 A_v C}{\epsilon_o \epsilon_r kT}\right)^{1/2} \quad (1)$$

the two charged systems had a double layer thickness (κ) on the order of 3 nm. Here k is Boltzmann's constant, T is the temperature, and ϵ_o and ϵ_r are the permittivity in a vacuum and the relative permittivity of the medium, respectively. The electronic charge is given by e, and A_v and C are Avogadro's number and the ionic concentration of the medium, respectively. In contrast to previous work using β-lactoglobulin (BLG) as an emulsifier (*26*), the double layer was thick enough to prevent flocculation in the creaming emulsion. However, once the cream layer became compressed, the droplets were forced closer together and the local interaction potentials of the two systems would have looked very different, particularly in light of the differences in surface mobility. This is because proteins are polyelectrolytes possessing a heterogeneous distribution of charge over their surface; therefore, when two protein molecules approach each other, the different charged groups will begin to interact on a local level, resulting in a local interaction potential that is very different from the long-range interaction which is controlled by the net charge on each molecule. Therefore, in an emulsion, the long-range interactions will be controlled by the net surface charge on the droplets, but short-range interactions will be influenced by the local distribution of charge. This is very different between the protein and the surfactant systems, in that it is possible for two protein-covered surfaces to form adhesive interactions at close range, but not for the surfactant system which possesses only negative charges. Calculation of the local pair potential for the two systems is difficult to do accurately for the protein system because of its polyelectrolyte/polyampholyte nature. However, it is clear that the short-range interaction is likely to be different.

As stated in the Introduction it has been shown that the rheological properties of dispersions are related to their size distribution through the Laplace pressure.[18] Because of this, we have endeavored to keep the droplet size distributions as similar as possible for all the emulsions and have measured the equilibrium interfacial tension of the systems used to make the emulsions. The interfacial tensions differed by a factor of 2 between the protein and surfactant systems; thus, the equations used by Bressy et al. (*16*) and those before (*10*) would predict a 2-fold difference in the droplet deformability and thus the bulk elastic modulus of the emulsions. This is not likely to have been increased significantly by the interfacial dilatational elastic modulus (E_d) between protein and surfactant systems. Even though the difference in E_d was approximately a factor of 25 at 0.1 Hz and less than 4% amplitude, this would equate to a decrease of less than 10% in the deformability of the protein-coated droplets and an insignificant decrease in the deformability of the surfactant-coated droplets. The higher elasticity would reduce drop deformation (*15*, *21*) and thus increase bulk elasticity (*17*) at the high phase volumes found in the cream layer, but the values of E_d could not provide the required decrease in deformability under the experimental conditions used. The bulk rheology (**Figures 3**−**5**) of the emulsion systems studied in detail shows that the elasticity was greater for the protein-stabilized system than for the surfactant system by approximately 30-fold. This clearly is not explained solely by changes in the Laplace pressure and suggests that other factors were involved.

There would seem to be two possible differences that might explain the creaming and rheological data. First, there are the differences in interfacial rheology shown in **Figure 5** and given in **Table 1**. These indicate that, in the concentrated region of the cream, the increased interfacial shear elasticity of the protein system would slow and possibly arrest the rearrangement of the droplets. This is because the hydrodynamic interactions between molecules at the interface and the solvent (Gibbs−Marangoni effect) have a direct impact on the velocity of solvent close to the surface. Thus, whereas a fluid interface will have little effect on the solvent velocity close to the interface, a highly viscoelastic interfacial layer will have a marked effect. This would then explain the differences in the maximum packing fractions for the two systems seen in **Figures 1** and **2**. However, there is clearly not a simple linear relationship between the interfacial and bulk moduli as the interfacial shear modulus shows a difference of more than 100-fold between the protein and surfactant systems. The second difference between the protein and surfactant interfaces is the close-range interaction. While surfactant-stabilized systems tend to have a repulsive interaction, even at close range, it has been shown using several different methods, such as thin films (*33*), magnetic chaining (*23*, *34*), etc., that protein-stabilized interfaces show a degree of adhesion. Thus, when two protein-covered interfaces are brought into close proximity, they tend to stick together. This has also been shown with concentrated emulsion systems which

did not spontaneously redisperse when diluted (*19*). The cream layer of the emulsions contains interfaces that are forced together by gravity, and the lack of Brownian diffusion observed in the cream layer of the protein-stabilized system suggests the drops were stuck together, at least more so than the surfactant-stabilized drops. In the case of the protein film and given the right conditions and sufficient time, there is also the possibility of disulfide bonds forming between proteins in the layers on neighboring droplets. This would certainly encourage the droplets that had been fixed in the cream layer for an extended period to stick together. Both of these differences are of course expressions of the same ability of protein films to form a cohesive network, both in three dimensions and in two. The interfacial rheology reflects this in two dimensions, and when the droplets are pressed close enough together, the interfacial films interact to form a more three-dimensional structure.

In summary, we have looked at an emulsion system in which the interfacial composition was varied between surfactant and protein but the long-range surface potential was maintained. Significant differences in the bulk rheology and creaming behavior were found. The protein system had a much higher bulk elastic modulus than the surfactant system and had a lower maximum packing fraction, which further accentuated the differences in bulk rheology. Careful control of the experimental conditions allowed us to demonstrate that droplet deformability, due to changes in interfacial tension and interfacial rheology, could not solely account for the huge changes in bulk rheology. This disparity was attributed primarily to differences in the close-range pairwise interaction but also to differences in the interfacial rheology of the two systems. Further experimental work is planned to try and quantify the specific contribution of these parameters to emulsion rheology and microstructure, and how we can control them to manipulate the flow and rheology of emulsion-based systems.

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