Interfacial Protein Networks and Their Impact on Droplet Breakup

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Conventional theories for predicting droplet disruption are based on a dimensionless capillary number and fail when a protein emulsifier is present at moderate concentration. In this study, droplet disruption is examined in simple shear and, in parallel, novel equipment is used to define conditions under which an interfacial stress-transmitting protein network forms at an oil/water interface. Capillary number predictions fail when a rigid interfacial protein network forms. Complete stress-strain curves, to high material deformation, are presented for these networks. This work demonstrates that droplets destabilized by interfacially adsorbed protein should be viewed as deformable capsules or cells surrounded by a stress-transmitting network. Deformation and disruption could then be predicted by existing theories for such systems, using the constitutive data provided by the stress-strain tests. Such an approach is expected to be superior to existing methods based solely on interfacial energy.

Introduction

Emulsion droplet size controls both the functionality of products and the efficiency and reproducibility of a range of processes. For example, interfacial area determines the productivity of some biocatalytic transformations (Hickel et al., 1999), the storage stability of drug emulsions (Zurowska-Pryczkowska et al., 1999), and the form and functionality of food products comprising multiple phases (Dickinson, 1992). Optimal emulsion functionality may be achieved at some optimum droplet volume as this determines the rates of settling or creaming (Walstra, 1996), and also the specific surface area available for adsorption of surface-active species. Therefore, the ability to predict droplet disruption in a given flow field is required to allow emulsion formulation with optimal droplet size, minimize energy input required for emulsification, and reduce the potential for damage of surface-active species in zones of excessive shear (Wolf, 1996).

Proteins adsorb at the air/water or the oil/water interface due to their amphiphilic nature (Graham and Phillips, 1979; Lu et al., 1999). This causes a reduction of the interfacial energy (Beverung et al., 1999) and may also result in a high degree of intermolecular interaction between the adsorbed protein molecules (Dickinson et al., 1985; Fourt, 1939; Petkov et al., 2000). While protein interfacial adsorption has been extensively studied, our ability to predict emulsion droplet size in even simple shear flow is lacking when protein is adsorbed at moderate concentration. For example, the work of Williams et al. (1997) has shown that the widely used approach of calculating a capillary number, and comparing with a plot of critical capillary number, fails when a moderate concentration ($> 0.01\ g \cdot L^{-1}$) of the globular protein β-lactoglobulin is present in the aqueous phase. The result is a droplet that is smaller than that predicted from the equilibrium interfacial tension using conventional theories. This indicates a droplet destabilizing mechanism that results in disruption at a lower shear rate than expected from interfacial energy correlations. In an earlier article, Janssen et al. (1994) showed that the presence of low molecular weight (non-protein) emulsifiers resulted in droplets that were always at least as stable as expected from capillary number predictions. In certain cases a greater shear rate (higher capillary number) was required, and this effect was phenomenologically characterized using an empirical interfacial tension greater than the equilibrium value. The authors attributed this effect to interfacial viscoelasticity, caused by the presence of an emulsifier, making droplet breakup more difficult.

In contrast, Williams et al. (1997) suggest that the failure of capillary number predictions at moderate to high β-

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lactoglobulin concentrations is due to the formation of an interfacial protein network. This assertion was supported by a sharp rise in the interfacial dilatational modulus when β-lactoglobulin is adsorbed from bulk concentrations in the range of 3×10^{-3} g·L^{-1} to 3×10^{-2} g·L^{-1} (Williams and Prins, 1996). The inability of existing theories to enable a prediction of droplet size suggests that a radical rethink of our view of emulsification is required. Such emulsions might, for example, be better considered as cells or as membrane-bound capsules. Such a view is not inconsistent with very early studies, such as those of Ascherson (1840), which drew analogies between cells and protein-stabilized emulsions.

Born et al. (1992) have modeled the disruption of mouse hybridoma cells under laminar shear stresses and compared the results with experimental data. The modeling technique assumes that the cell can be described as an elastic membrane surrounding an incompressible fluid, and that the cell membrane tension corresponds to the interfacial tension of the droplet. However, this value is allowed to change during the cell deformation that occurs before disruption. Despite the fact that the dynamic behavior of the cells is neglected, the experimental and modeling results agree to within 30%. A more fundamental approach based on capsule theory has been proposed by Barthes-Biesel (1991), who summarizes the problem by stating that, "The capillary number definition is based on a single constant value of a shear elastic modulus and does not contain any information on the rheological behavior of the membrane. This raises the question of the appropriateness of the capillary number to measure the ratio of the elastic to viscous forces for non-Hookean materials." The author develops a model based on normal and tangential stress balances that could be combined with a full constitutive equation describing the interfacial or membrane response to stress. Unfortunately, application of this fundamental theory to the prediction of droplet size in simple shear is limited by a lack of such constitutive equations for interfacial protein networks.

In the present study, we address the problems highlighted above through two specific objectives. First, we investigate the destabilizing effect caused by moderate to high concentrations of β-lactoglobulin, and show conclusively that an interfacial network capable of lateral stress transmission can be formed at the oil/water interface. Secondly, we determine the full stress-strain constitutive behavior of an interfacial protein network to high deformation. Such data provide the basis for a full material constitutive equation for subsequent use in the Barthes-Biesel formalism. These objectives are achieved through the use of novel equipment: the Cambridge Interfacial Tensiometer (CIT). The CIT is capable of generating full stress-strain plots for protein networks adsorbed at the oil/water interface, allowing the generation of an experimentally determined constitutive equation (Jones, 2002; Jones and Middelberg, 2002a). The ability to generate a complete material constitutive equation enables the theory of Barthes-Biesel (1991) to be employed to predict droplet breakup during the application of fluid shear. Such information could be used to optimize emulsion processing conditions, or to facilitate the design of biomolecules having the most favorable characteristics for the creation and stabilization of emulsion-based products. This approach would enable emulsion product optimization through the combined processes of molecular design and hydrodynamic specification.

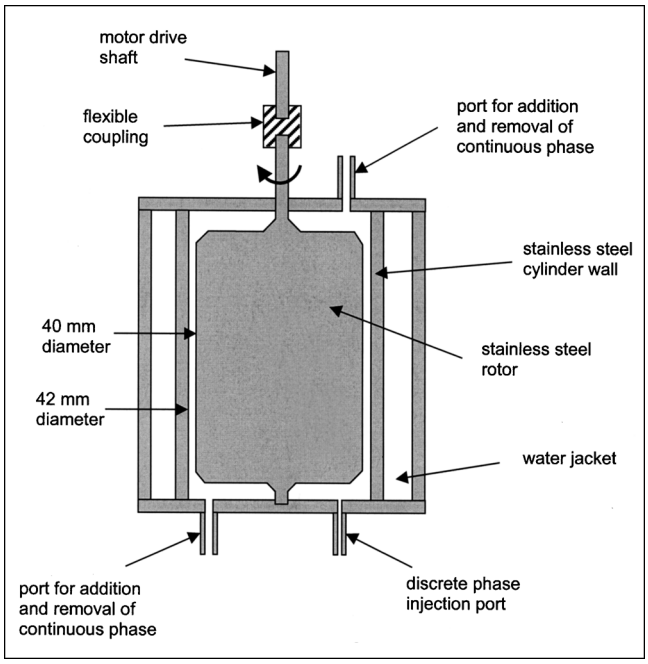

Figure 1. Couette shear apparatus.

Materials and Methods

Droplet disruption and sizing

Droplets were disrupted using custom-built Couette shear apparatus shown in Figure 1. The equipment consists of a rotating cylindrical bob surrounded by a stationary outer cylinder, creating approximate simple shear in the annulus due to the small separation (1 mm) relative to the cylinder diameter (40 mm). The continuous phase was 80% glycerol (BDH Laboratory Supplies, U.K.) solution in ultrapure water (resistivity $\geq 18.2\times10^{4}$ Ω·m, MilliQ Gradient System, Millipore, U.K.), with varying concentrations of β-lactoglobulin ($3\times$ crystallized and lyophilized, Sigma, U.K.). Silicone oil (AS4, $\mu = 6$ mPa·s, Fluka, U.K.) was used as the dispersed phase. Both the continuous and discrete phases are Newtonian at the chosen experimental conditions. The two larger ports shown in Figure 1 were used to fill and empty the device using PVC tubing (Tygon-Food, Cole Parmer, U.K.) and a peristaltic pump. The oil phase was injected after the apparatus had been filled with glycerol solution and was withdrawn with the continuous phase for droplet size measurements. The continuous phase was very slowly pumped backwards and forwards during a 5 min shearing phase, during which the oil droplets experienced multiple passes through the narrow annulus. The pump was placed such that the dispersed phase never passed through the pump itself. Vertical motion of the oil droplets through the annulus was very low relative to the rotation speed of the inner cylinder, and is therefore neglected in the calculation of shear rate.

After 5 min of shearing, a sample of the continuous phase containing the disrupted oil droplets was removed for particle sizing using a Laser Diffraction Coulter Counter (LS230,

Beckman Coulter, U.K.). The emulsion sample required dilution with β-lactoglobulin solution (0.25 $g \cdot L^{-1}$) as the continuous phase viscosity far exceeded the 5 mPa·s limit of the laser particle sizer. Coalescence times were determined experimentally and found to be greater than the measurement time scale due to the lean nature of the diluted emulsion and the high concentration of stabilizing protein. The droplet diameter occurring with the maximum frequency was used in all further calculations.

Calculation of the capillary ratio

The capillary number is defined as

$$\Omega = \frac{G \cdot \mu \cdot d}{\gamma}$$

where G = shear rate (s^{-1}), μ = continuous phase viscosity (Pa·s), d = droplet diameter (m), and γ = the equilibrium interfacial tension ($N \cdot m^{-1}$).

Interfacial tension for a droplet of silicone oil in 80% glycerol solution was determined using a pendant drop apparatus (DSA10, Kruss, Germany). The interfacial tension was measured for all protein concentrations used for droplet disruption at the same interface age (5 min). An interface age of 5 min was chosen as this is equal to the total shearing time and, therefore, corresponds to the lowest value of interfacial tension likely to be reached for the oil droplets in the Couette shear device. However, further changes in the interfacial tension may take place after the 5 min period, because a true equilibrium value of interfacial tension for protein solutions is rarely reached (Beverung et al., 1999).

The capillary ratio is defined as the critical capillary number for the experimental conditions divided by the critical capillary number predicted from a plot of critical capillary number vs. viscosity ratio (de Bruijn, 1989). In effect, it is the observed value divided by the expected value for a clean system with the same interfacial tension. In these experiments droplets were generated at the maximum stable droplet size. Therefore, the capillary number calculated from the experimental conditions is equivalent to the critical capillary number.

Mechanical testing of protein networks

The Cambridge Interfacial Tensiometer (CIT) is capable of measuring the force response of a protein network adsorbed at the oil/water interface and subjected to a uniaxial strain. Full details of the equipment design, and its validation, are presented elsewhere (Jones, 2002; Jones and Middelberg, 2002a). Previous studies have confirmed that the established micro-mechanical parameters are not a function of the selected design parameters (Jones, 2002). In summary, two identical silica T-bars were located at the oil/water interface (Figure 2). The T-bars were 50 mm long, 400 μm in diameter, had an initial separation of 1 mm, and were constructed from the cladding and core of silica-silica fiber optic cable (Type FT-400-UMT, Thorlabs Inc., NJ). Two silica fibers were joined to create a T-bar using molten polypropylene from 10^{-6} L pipette tips (Fisher, U.K.). One T-bar was connected to a solid-state motor (Inchworm, Burleigh Instruments Inc., NY) providing linear displacement over a wide range of operating speeds and incorporating an internal position encoder with 50 nm resolution. The other T-bar was connected to a sensitive force transducer (Model 403A, Aurora Scientific Inc., Ontario, Canada) to measure forces transmitted laterally through the protein network in the plane of the interface. The CIT is insensitive to interfacial tension when operated in this mode (Jones, 2002).

Figure 3 shows a cross-section of the CIT instrument and the T-bar location at the oil/water interface. In these tests, the oil phase was octane (Sigma-Aldrich, U.K.) and the aqueous phase was a solution of β-lactoglobulin (3×crystallized

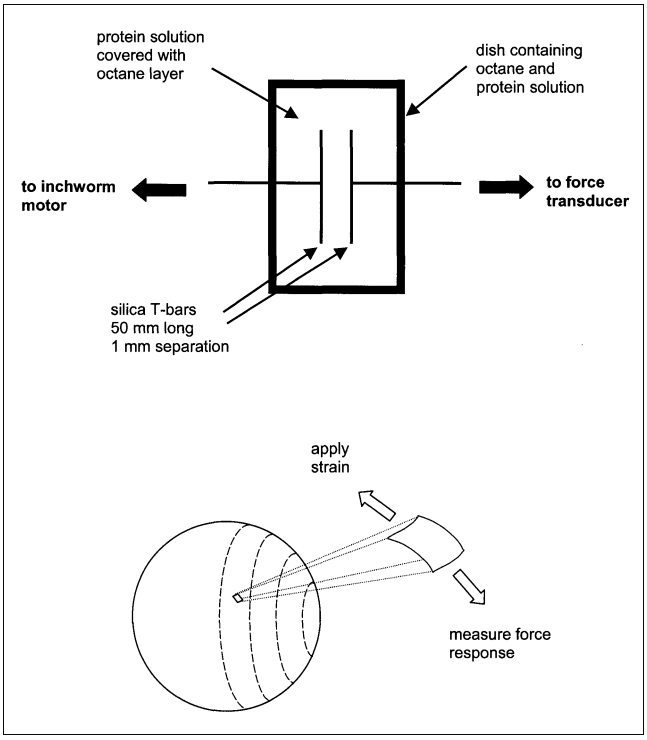

Figure 2. CIT (plan view) and use of a material constitutive equation to predict droplet disruption.

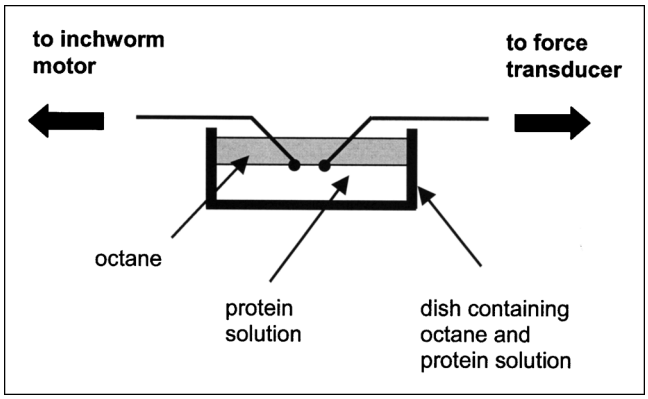

Figure 3. CIT, side view.

and lyophilized, Sigma-Aldrich, U.K.) or β-casein (lyophilized, Sigma-Aldrich, U.K.) dissolved in a PBS buffer (137 mM NaCl, 2.7 mM KCl, 12 mM Na_2HPO_4, 1.8 mM KH_2PO_4, adjusted to pH 7.4 with HCl) made using ultrapure water. The octane was passed 10 times through a 25 cm column of silica gel (35–70 mesh ASTM, Fluka, U.K.) that had been roasted for 14 h at 290°C before use to remove any surface-active contaminants. The silica T-bars were cleaned before use by soaking for 30 min in "Nochromix" glass cleaning reagent (Godax Laboratories Inc., MD) dissolved in concentrated HCl (Fisher, U.K.). The T-bars were then washed to a pH neutrality in ultrapure water and derivatized using an amine silane. The derivatization process involved submerging the T-bars in a solution consisting of 50×10^{-3} L-toluene, 50×10^{-6} L (3-aminopropyl) triethoxysilane, 50×10^{-6} L of ultrapure water and 5×10^{-6} L of Tween20 and shaking for 20 min before extensive washing with ultrapure water. All reagents for this derivatization step were purchased from Sigma-Aldrich, U.K. Before each test, the silica T-bars and dish were rinsed *in situ* three times with 100×10^{-3} L of ultrapure water. This was followed by washing with acetone, then ethanol, and finally rinsing another three times with ultrapure water.

The CIT was operated by moving the T-bar connected to the motor from its original separation of 1.0 mm to 11.0 mm (1,000% strain) at a speed of 200×10^{-6} m·s^{-1}, and then immediately reversing the direction of travel to return the bar to its starting position. The procedure therefore has two stages, the first being a tensile testing phase, and the second a compressive test. Force is normalized with bar length to give an interfacial stress. Bar separation is normalized with initial separation to give a strain, allowing the creation of a stress-strain plot for the interfacially adsorbed protein network.

Production of a full stress-strain plot results in a loss of structural integrity of the interfacially adsorbed material. The CIT, however, can be operated in a low strain (5%) minimally invasive cyclic mode, allowing lateral force transmission through a single protein network to be recorded as a function of time. In these tests the separation of the T-bars was increased to 1,050 μm from 1,000 μm and then returned to the original position at a linear speed of 200×10^{-6} m·s^{-1}. The increase in tensile force resulting from the 5% tensile displacement was recorded. Cycle frequency was 10 cycles per min.

Results and Discussion

The results shown in Figure 4 demonstrate that the capillary ratio is reduced below unity when the β-lactoglobulin concentration in the aqueous phase is greater than 10^{-2} g·L^{-1}. This clearly shows that the droplet destabilizing mechanism observed by Williams et al. (1997) can be reproduced in a new system with very different physical properties. The capillary ratio indicates the ratio of the experimentally determined critical capillary number divided by that predicted from a plot of critical capillary number vs. the viscosity ratio of the dispersed and continuous phases. This is effectively the observed value of the capillary number divided by the expected value. The actual droplet size obtained is reduced below that predicted by the interfacial tension (capillary ratio < 1) when

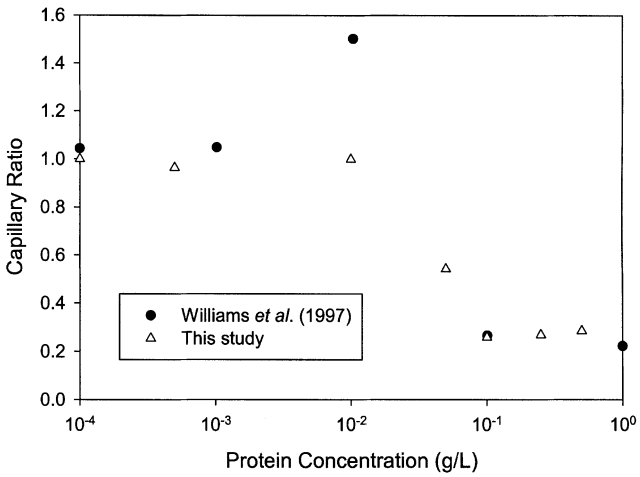

Figure 4. Capillary ratio as a function of β-lactoglobulin concentration.

Filled circles show data of Williams et al. (1997).

the bulk β-lactoglobulin concentration exceeds 10^{-2} g·L^{-1}.

In this study, the dispersed phase was silicone oil and the continuous phase was 80% glycerol solution. The viscosity of the continuous phase was increased to provide a sufficient shear force to disrupt the oil droplets. Glycerol was used to thicken the aqueous phase and silicone oil was chosen as the dispersed phase, as both show Newtonian behavior under the experimental conditions. Droplet size in this work is in the range of 15 μm–65 μm. Williams et al. (1997) inverted the phases using silicone oil as the continuous phase and an aqueous drop. Corn syrup was used to increase the viscosity of the aqueous phase and the drops were around 1 mm in diameter. The same destabilizing effect was observed despite such differences in experimental procedure, indicating the generic effect of high concentrations of β-lactoglobulin in modifying droplet disruption. Williams et al. (1997) also investigated the effect of β-casein and found that no such modification of the droplet disruption mechanism could be observed, beyond the changes expected due to reduced interfacial tension. Therefore, mechanical testing of protein networks adsorbed at an oil/water interface was performed, seeking to relate the protein network properties to droplet disruption characteristics. Two protein concentrations were chosen, one above and one below the β-lactoglobulin concentration shown to modify droplet disruption in a way that cannot be accounted for by the interfacial tension alone.

The CIT measures lateral force transmission in the plane of the flat interface. The equipment is, therefore, insensitive to interfacial tension itself, and essentially detects lateral protein-protein interactions of the adsorbed species. Previous work has shown that the ensemble properties can be predicted from single-protein unfolding studies using atomic force microscopy (Jones and Middelberg, 2002b). The T-bars initially begin with a separation of 1.0 mm, and one is moved at a speed of 200×10^{-6} m·s^{-1} until a total separation of 11.0 mm (1,000% strain) has been achieved. This is the tensile section of the test procedure and results in a positive (tensile) force being registered by the opposing silica T-bar.

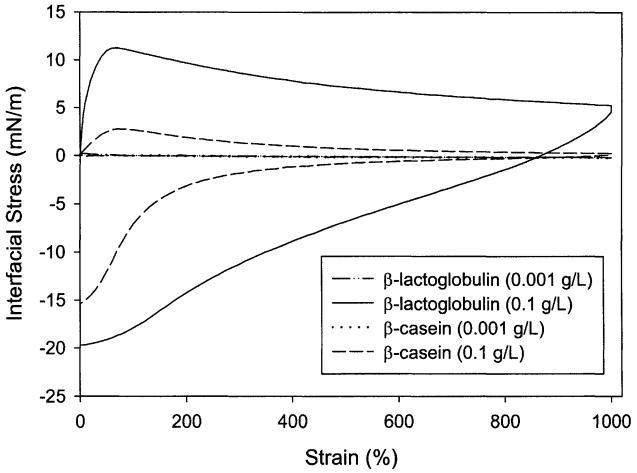

Figure 5. Average stress-strain plots for protein films adsorbed at the octane/water interface from solution in PBS buffer (pH 7.4).

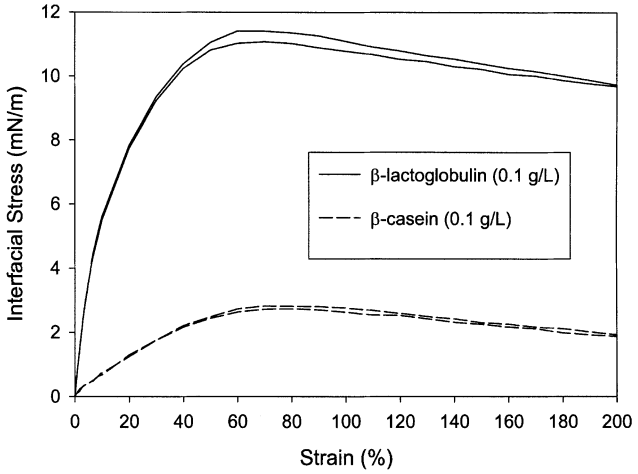

Figure 6. Stress-strain plots for β-lactoglobulin and β-casein films adsorbed at the octane/water interface from a 0.1 $g \cdot L^{-1}$ protein solution in PBS buffer (pH 7.4).

The direction of travel is then immediately reversed and the bar separation reduced back to 1 mm to provide information about the response of the protein network to a compressive load.

Figure 5 shows full stress-strain plots for β-lactoglobulin and β-casein adsorbed at the octane/water interface. The stresses transmitted through the protein film, in both tension and compression, are greatest when the bulk aqueous phase contains 0.1 $g \cdot L^{-1}$ β-lactoglobulin. In this case the curve shows a very steep initial response where the network shows a high degree of rigidity or high interfacial elasticity modulus. The correlation with atomic force microscopy data in this region indicates that the forces measured by the CIT represent the average force required to induce conformational changes in the adsorbed protein molecules. The rigidity decreases at higher strains and maximum interfacial stress is reached at around 75% strain. This is most likely a result of flaws generated in the interfacial network, as intermolecular bonds are disrupted by the imposed strain. This is then followed by a long plateau region where the network shows considerable ability for "repair," probably as a result of adsorption of further protein molecules at exposed regions of the oil/water interface (Jones and Middelberg, 2002b). When the direction of travel is reversed, the tensile stress is immediately reduced. This stress becomes negative (compressive) very early in this compressive section of the test, providing further evidence of protein adsorption during the tensile procedure. The very high compressive forces registered probably indicate the compression of multiple protein layers at this point in the test.

The results for 0.1 $g \cdot L^{-1}$ β-casein show a much lower initial elasticity modulus and peak interfacial stress than obtained for 0.1 $g \cdot L^{-1}$ β-lactoglobulin. The peak interfacial stress for β-casein is not reduced by the same margin as the initial elasticity modulus. This demonstrates that the degree of intermolecular cohesion of β-casein molecules adsorbed at the interface is not as low, as has been previously suggested (Williams and Prins, 1996) and that a protein network structure is still formed, albeit a weaker structure than for globular β-lactoglobulin. At a protein concentration of 10^{-3} $g \cdot L^{-1}$, neither β-casein nor β-lactoglobulin form a cohesive network structure capable of transmitting a significant force laterally through the adsorbed protein layer. The curves on Figure 5 represent the average of two tests at each set of experimental conditions. For a protein concentration of 0.1 $g \cdot L^{-1}$, where force transmission is significant, both runs have been plotted in the low strain regime in Figure 6. This shows the reproducibility of the technique and highlights the differences in the initial elasticity modulus (initial gradient of stress-strain plot) between the two types of protein network.

The stress-strain plots shown in Figures 5 and 6 were all performed after a 5 min aging time and, therefore, can provide little insight into the dynamics of the formation of interfacially adsorbed protein networks. To solve this problem, the CIT can be operated in a cyclic, low strain mode that provides an ability to probe lateral force transmission through the protein network in a minimally invasive manner. The T-bar separation is increased by 5% at a speed of 200×10^{-6} $m \cdot s^{-1}$. The direction of travel is then reversed to return the T-bar to its original position and the cycle is repeated 10 times per min. The results for duplicate runs of such tests are provided in Figure 7. The inset shows relevant data for β-lactoglobulin from the capillary ratio plot. The results show a striking difference between data for 0.1 $g \cdot L^{-1}$ β-lactoglobulin and the other data. The protein network formed from a high bulk concentration of β-lactoglobulin shows a much higher level of force transmission at low strain than that obtained for low protein concentrations or for 0.1 $g \cdot L^{-1}$ β-casein.

Figure 7 clearly shows that while there is some degree of enhancement in force transmission as the film ages over the 5 min period, a rigid protein network is formed immediately as the aqueous and oil phases meet. This raises a very important point of experimental procedure, as it appears that protein network properties are determined, in the main part, by the initial adsorption of protein molecules at the clean fluid/fluid interface (Jones, 2002). In these tests, the octane is placed in

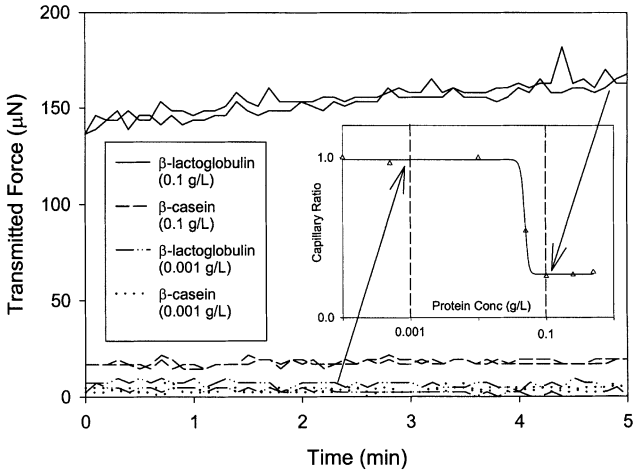

Figure 7. Lateral force transmission through protein films at low strain (5%).

Proteins adsorbed at the octane/water interface from solution in PBS buffer (pH 7.4). Inset shows capillary ratio transition from Figure 4, with data points shown as Δ and the solid line drawn as a guide only.

the dish before the protein solution is added. The aqueous phase sinks below the octane as it is slowly poured into the dish, and the protein molecules adsorb at a clean octane/water interface. This contrasts with interfacial rheology experiments of other workers who have poured an oil phase over the top of an aqueous protein solution (Dickinson et al., 1985; Williams and Prins, 1996), thereby modifying the behavior of protein molecules that originally adsorbed at the air/water interface.

The fact that network rigidity is relatively constant over a 5 min period explains the observation made in this study that shearing times longer that 5 min have no significant effect upon the size distribution of the resulting emulsion. This is in agreement with Williams et al. (1997) who report that variation in the time taken to reach the critical capillary number (within the range 1–20 min) had no effect on the results. If the dynamics of the formation of a protein network with high elasticity modulus are not important, then both modes of CIT operation can be used to predict whether the capillary number approach is a suitable tool to analyze droplet disruption.

Data extracted from full stress-strain plots are presented in Table 1. This table also contains the elasticity modulus obtained from an analysis at 1% strain where the response of adsorbed β-lactoglobulin networks has been shown to be approximately linear (Jones and Middelberg, 2002b). The network rigidity to 1% strain shows the same "on/off" behavior as the destabilizing mechanism occurring during droplet shearing. The initial elasticity modulus for β-casein and the lower β-lactoglobulin concentration are very small relative to that obtained for 0.1 $g \cdot L^{-1}$ β-lactoglobulin, following exactly the same pattern as the droplet disruption experiments summarized in Figure 4. These results strongly suggest that the formation of a protein film with a high initial elasticity modulus causes the destabilization of an emulsion droplet undergoing shear. This partially explains the widespread use of β-lactoglobulin as an emulsification agent in foodstuffs and suggests that droplet disruption will be enhanced in the presence of emulsifiers capable of forming a rigid interfacial network. The CIT has a unique ability to detect the formation of a rigid protein network, unobscured by interfacial tension effects, and is therefore a valuable tool to predict whether droplet disruption is dominated by interfacial tension effects or by interfacial network formation. The instrument may be used to rapidly test new molecules for their likely efficacy in facilitating emulsification.

Table 1. Mechanical Properties Determined by CIT for β-lactoglobulin and β-casein Protein Networks Adsorbed at the Octane/Water Interface

	β-Lactoglobulin		β-Casein	
Protein Conc. (g/L)	0.001	0.1	0.001	0.1
Max. interfacial stress (10^{-3} N/m)	0.2	11.2	0.2	2.8
Min. interfacial stress (10^{-3} N/m)	−0.2	−19.7	−0.2	−15.4
Interfacial elasticity modulus (1% strain, 10^{-3} N/m)	9.2	88.6	5.9	12.4
Force transmission (5% cyclic strain, 10^{-6} N)	0.0	165.3	2.4	19.2

Most attempts to model droplet disruption neglect all effects of emulsifiers other than their direct effect of modifying the equilibrium interfacial tension. Such approaches make two implicit assumptions. The first is that stresses are transmitted without loss between the internal and external fluid phases and, secondly, that the local interfacial tension is equal to the equilibrium interfacial tension. Breakup has been predicted to occur when the droplet capillary number exceeds some critical value. This situation occurs when the stabilizing Laplace (interfacial tension) forces can no longer balance the viscous forces, particularly near the waist of an elongated liquid droplet (Barthes-Biesel, 1991). Janssen et al. (1994) have shown that, in the presence of small molecule surfactants, droplet breakup can be more difficult than that predicted from the equilibrium interfacial tension (that is, capillary ratios > 1). The authors characterize this effect by using an effective interfacial tension that is greater than the equilibrium interfacial tension, to account for the imbalance created by nonuniform surfactant distribution at the interface caused by droplet deformation. Regions of interface dilation (such as the waist of elongated droplet) have a lower interfacial concentration of adsorbed surfactant. This results in a higher localized value of interfacial tension, generating a force that resists further deformation and makes droplet disruption more difficult. The results shown in Figure 4 indicate a qualitatively different effect, resulting in a droplet destabilizing mechanism that cannot be explained by the presence of a nonzero dilatational elasticity modulus (change in interfacial tension with area).

Fisher et al. (1978) have compared the deformation and motion of liquid drops with that of red blood cells. The results obtained indicate that the mean interface rotation rate is independent of the viscosity ratio of the dispersed and continuous phases for red blood cells, due to the presence of the cellular membrane. These results are similar to those obtained by Williams et al. (1997) for high concentrations of β-lactoglobulin where solid body rotation was observed and circulation rate was found to be independent of the viscosity

ratio. The results for 0.1 $g \cdot L^{-1}$ β-lactoglobulin shown in Figure 5 strongly suggest that, under these conditions, a suspended fluid droplet is bounded by a rigid interfacial network capable of transmitting a force in the plane of the interface. Lateral interactions between interfacially adsorbed protein molecules result in the formation of a rigid interfacial network. As the number of adsorbed protein molecules rises, a critical interfacial concentration is reached where the individual adsorbed protein molecules are no longer able to move freely in the plane of the interface. This changes the droplet disruption regime from one dominated by dynamic interfacial energy considerations to a network-controlled regime. Thin membrane analysis performed by Barthes-Biesel (1991) predicts that, under such network forming conditions, the internal liquid undergoes bulk solid body rotation and is not sheared. "As a consequence, the externally applied tangential stresses are absorbed by the membrane deformation process." Effectively, departure from an interfacial energy dominated regime, where stresses are transmitted undiminished across the fluid-fluid interface, changes the boundary conditions of the problem description. The results of this study suggest that the formation of a rigid network allows forces to be transmitted laterally in the plane of the interface, increasing localized network stress and causing droplet destabilization. This mechanism explains the ability of the globular protein β-lactoglobulin to destabilize liquid droplets by forming a rigid protein film when adsorbed at the interface in high concentrations.

Evidence of inhomogenous stress propagation through adsorbed protein films can be found in the results of some elegant experiments performed by Langmuir and Schaefer (1939) to investigate the degree of protein intermolecular cohesion. The experiments involved spreading a protein monolayer on the air-water interface and expanding the central region with a spreading oil. Two distinct pattern types were formed by different proteins, a star-like pattern and a smooth circular pattern. These results are reproduced in Figure 8. In an earlier article Schaefer (1938) made the observation that, "protein monolayers which produce expansion patterns of the star-like form are, in general, of the type described by Hughes and Rideal (1932) as a gel structure." Langmuir and Schaefer (1939) noted that protein films capable of providing a high degree of damping to a disc oscillating in the interfacial region tended to form star shaped patterns. Such observations

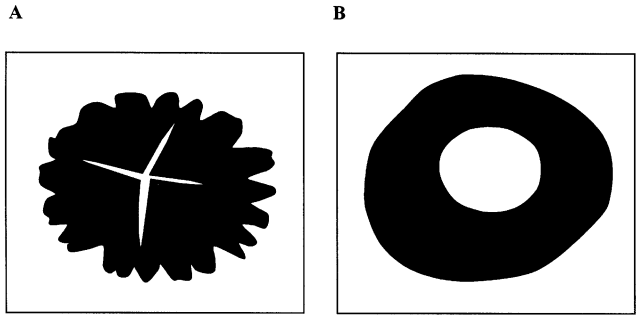

Figure 8. Starlike expansion pattern A (egg albumin, pepsin, tobacco seed globulin) vs. smooth circular expansion pattern B (insulin, casein).
Redrawn from data by Langmuir and Schaefer (1939).

provide evidence that protein films with a solid-like (rigid) structure can cause nonuniform stress distribution resulting in a localized failure of the protein network, in an analogous fashion to crack propagation in conventional materials. There is a striking visual similarity between the work of Langmuir and Schaefer (1939) and recent work by Mackie et al. (1999) who investigated the displacement of protein from the air/water interface by the nonionic surfactant Tween20. The surfactant was found to adsorb at defects in the protein network, whereupon the protein network was compressed by the growing surfactant nucleation sites. The authors noted that, "stress propagation through β-casein films is homogenous resulting in the growth of circular surfactant domains," while "β-lactoglobulin and α-lactalbumin form stronger networks and stress propagation is restricted resulting in the growth of irregular (fractal) surfactant domains."

Protein network formation appears to change the difficulty and mechanism of droplet disruption by causing a discontinuity in the tangential stresses across the interface. In the case of a liquid or emulsion droplet such a discontinuity is not present, as any film existing between the two phases merely transmits tangential stress from one fluid to the other. Therefore, oil droplets bounded by a rigid protein network may be better considered as capsules or even model mammalian cells where the membrane and internal cell properties are more easily characterized. Zhang et al. (1992) have considered the bursting of mouse hybridoma cells under mechanical stress using a micromanipulation technique, and subsequently modeled the system as an elastic membrane surrounding an incompressible fluid. The membrane bursting tension for the cells was found to be 1.8×10^{-3} $N \cdot m^{-1}$. This compares favorably with the $2.8-11.2 \times 10^{-3}$ $N \cdot m^{-1}$ range of maximum tensile stress transmission found in this study for β-casein and β-lactoglobulin, respectively, at 0.1 $g \cdot L^{-1}$ protein concentration. In the same year Born et al. (1992) developed a model to predict disruption of animal cells by laminar shear stresses. The cells were modeled as an emulsion of two immiscible liquids using equations proposed by Taylor (1934) and assuming that cell membrane tension corresponds to droplet interfacial tension. The cell membrane tension changes during the deformation using the approach of Born et al. (1992), but the dynamic behavior of the cells is ignored and, therefore, the actual, or apparent, cell or droplet viscosity is neglected. However, the model achieved agreement with the experimental cell disruption data to within 30% and, therefore, demonstrates the useful predictive capability of this approach.

For a viscoelastic membrane, the material properties depend on several parameters, so a model based upon a full description of the interface will have improved accuracy over a model using a single value of bursting membrane tension. Barthes-Biesel (1991) has considered the influence of interfacial properties on the deformation and breakup of capsules in shear flow. The author develops a model based on normal and tangential stress balances coupled with a constitutive equation to describe the interfacial properties. The CIT is capable of generating full stress-strain plots at varying rates of strain for protein networks adsorbed at the oil/water interface, allowing the generation of an experimentally determined constitutive equation for the droplet interface (Figure 2). The exclusive ability of the CIT to generate an experimen-

tally derived constitutive equation for an interfacial protein network will thus enable *a priori* prediction of droplet disruption to be made using numerical methods.

The data and supporting literature presented in this article strongly suggest that correlations based on interfacial energy alone are unsuitable in the case of a rigid interfacial network, and that the system is more accurately considered as a capsule bounded by a viscoelastic membrane. It seems this was first realized by Ascherson (1840) when he believed he had created momentarily viable cells by producing protein stabilized emulsions. Although the assertion that the emulsion droplet could be directly equated with living cells is incorrect, the analogy is useful as emulsion droplets surrounded by protein networks could be considered as "ghost" cells. Under these circumstances, independent and direct determination of the mechanical properties of the "cell membrane" is possible using the CIT. This would facilitate the development of further models to predict animal cell damage or disruption in more complex flow fields found in bioreactors, without additional complications arising from biological variability or cell age.

Conclusions

Droplets of silicone oil in glycerol solution have been successfully disrupted in a flow pattern that approximates simple shear. At low β-lactoglobulin concentrations (< 0.01 g$\cdot$L^{-1}), droplet disruption can be predicted from the capillary number correlation using the shear rate, interfacial tension, and bulk phase viscosity. At β-lactoglobulin concentrations greater than 0.01 g$\cdot$L^{-1}, such an approach fails, and the maximum stable droplet size and capillary number are reduced below the expected value. This is shown in Figure 4, where the capillary ratio is less than 1 at the higher β-lactoglobulin concentrations. Our work is in good agreement with that of Williams et al. (1997) on larger single droplets where silicone oil was used as the continuous phase and a corn syrup solution was the dispersed phase. This suggests that the ability of β-lactoglobulin to modify droplet breakup at high bulk concentrations is a generic effect that is not system specific.

We have used custom built equipment (CIT) to probe the response of adsorbed protein layers to uniaxial strain in a way that eliminates the contribution from equilibrium interfacial tension. We believe such a technique to be required as the shearing results suggest that, at high β-lactoglobulin concentrations, use of correlations based on the interfacial tension, such as the capillary number, are incapable of predicting droplet disruption even if allowance is made for the dilatational elasticity modulus. The CIT also tackles the general paucity of literature regarding mechanical properties of protein films adsorbed at the oil/water interface when compared to the air/water interface, as the experimental technique is easily applied at both types of interface. The results show that, at high concentrations of β-lactoglobulin (0.1 g$\cdot$L^{-1}), the adsorbed molecules can transmit a force in the plane of the interface. Our results indicate the formation of a rigid β-lactoglobulin network at the octane/water interface at 0.1 g$\cdot$L^{-1} bulk protein concentration. Networks capable of transmitting significant forces laterally in the plane of the interface are not formed at the octane/water interface for 0.001 g$\cdot$L^{-1} β-lacto-globulin or 0.001 g$\cdot$L^{-1} β-casein. At 0.1 g$\cdot$L^{-1} concentration, β-casein is capable of forming a cohesive network, but with a tensile rigidity much below that obtained for the same concentration of β-lactoglobulin.

Comparison of the droplet shearing data and those obtained using the CIT shows that more efficient droplet disruption occurs when a rigid interfacial network is formed. We suggest that a rigid interfacial network, capable of transmitting forces laterally in the plane of the interface, destabilizes the liquid droplet by increasing localized network stress. Cyclic operation of the CIT provides a clear and quick test to show the presence of a rigid protein network, and can, therefore, indicate the suitability of the capillary number in the prediction of droplet disruption. We have established the CIT as a useful predictive tool for determining the most appropriate theory for the prediction of droplet size after disruption in a known flow field. The richness of features in a full stress-strain plot of an interfacially adsorbed protein make the CIT an ideal instrument to provide detailed information regarding the macroscopic network properties that will be useful for the molecular design and testing of a wide range of emulsifiers and other interfacially adsorbed biomaterials. Furthermore, the full stress-strain plots generated by the CIT provide a network constitutive equation which can be coupled to the fluid equations, enabling *a priori* computational modeling of droplet deformation and disruption, as proposed by Barthes-Biesel (1991).

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