Bulk Elasticity of Concentrated Protein-Stabilized Emulsions

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We prepared concentrated quasi-monodisperse hexadecane-in-water emulsions stabilized by various proteins, and we investigated their rheological properties. Some protein-stabilized emulsions possess remarkably high elasticity, being at the same time considerably fragile—they exhibit coalescence at yield strain and practically do not flow. The elastic storage modulus G' and the loss modulus G'' of the emulsions were determined for different oil volume fractions above the random close packing. The results for G' were normalized by the capillary pressure of the nondeformed droplets and further compared with literature data obtained on emulsions stabilized by classical surfactants. Surprisingly, the dimensionless elastic modulus $G'/(\sigma/a)$ (σ being the interfacial tension and a being the drop radius) obtained for emulsions stabilized by different proteins does not collapse on a single master curve, being almost always substantially higher than the corresponding values obtained for equivalent surfactant-stabilized emulsions. Furthermore, we found $G'/(\sigma/a)$ to be correlated (for a given oil volume fraction) to the dilatational elastic modulus ϵ of the protein layer adsorbed on the droplets. The osmotic equation of state of our emulsions was determined experimentally and was found to be almost identical with the one obtained for samples stabilized by classical surfactants. This provided a hint that the emulsions' elasticity is not connected with any specific properties of the bulk continuous phase. We were also able to produce elastic biliquid foams, containing practically no water, by free evaporation of the aqueous phase. This was possible most probably because of the formation of a relatively thick and rigid protein layer(s) that protects the fat globules against coalescence.

1. Introduction

Emulsions are among the most frequently used colloidal systems, largely present in areas such as foods, cosmetics, pharmacy, oil recovery, painting, etc. Everyday routine provides numerous examples for protein-stabilized emulsions, e.g., milk, mayonnaise, yogurt, table spreads, some cheeses, etc. It is evident that the properties of these complex systems are of both technological and academic interest.[1−4]

Emulsions are dispersions of deformable droplets; hence, different interfacial configurations could be realized as a function of the oil volume fraction. Much effort has been spent in studying the rheology of relatively diluted emulsions (oil volume fraction below 40−45%). It was demonstrated[2] that the transition from a nonflocculated to a flocculated state is accompanied by a change in the flow properties of emulsions. Nonflocculated samples generally obey a Newtonian flow, while flocculated emulsions show shear-thinning behavior. At low volume fraction ϕ, emulsions consist of unpacked spherical droplets. Generally they do not exhibit shear rigidity, and they are dominantly viscous like the suspending fluid. However, because of the deformability of the droplets, emulsions may be concentrated up to volume fractions much higher than the volume fraction ϕ^*, corresponding to the close packing of an equivalent suspension of hard spheres. For randomly packed monodisperse spheres, $\phi^* = 0.64$.

Emulsions are systems comprised of fluids, but at a high internal phase volume fraction (at which the droplets can no longer pack without deforming), they become remarkably rigid and resemble an elastic solid. As pointed out by Princen[5] and Mason et al.,[1] the considerable elasticity of the concentrated emulsions exists because the repulsive droplets have been compressed by an external osmotic pressure, Π. Indeed, two droplets forced together will begin to deform before their interfaces actually touch, because of the intrinsic repulsive interactions between them. Thus, emulsions minimize their total free energy by reducing the repulsion (which may have different origins) at the expense of creating some additional surface area by deforming the droplet interfaces. The necessary work to deform the droplets is done through the application (by any means) of an external osmotic pressure Π, and the excess surface area of the droplets determines the equilibrium elastic energy stored at a given osmotic pressure. Additional excess surface area created by a perturbative shear deformation determines the elastic shear modulus, $G'(\phi)$. Although Π and G' represent fundamentally different properties, they both depend on the degree of droplet deformation and therefore on ϕ. For a detailed review, the reader is directed to ref 1.

The first quantitative study on the rheological properties of monodisperse emulsions was performed by Mason et al.[1] They investigated silicon oil-in-water emulsions stabilized by sodium dodecyl sulfate (SDS). The elastic shear modulus was found to increase by nearly 4 decades as ϕ increases from 0.1 to about 0.9 and to exhibit a universal dependence on the effective oil volume fraction, when scaled with the Laplace pressure of the nondeformed droplets. They provide an uncontestable proof of the original work of Princen,[5,6] who first predicted the scaling of G' with the capillary pressure, σ/a (σ being the interfacial

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(1) Mason, T. G.; Krall, A. H.; Gang, H.; Bibette, J.; Weitz, D. A. In *Encyclopedia of Emulsion Technology*, Becher, P., Ed.; Marcel Dekker: New York, 1996; Vol. 4, p 299 and references therein.

(2) (a) Dickinson, E.; Golding, M. *J. Colloid Interface Sci.* **1998**, *197*, 133. (b) Dickinson, E.; Chen, J. *J. Agric. Food Chem.* **1998**, *46*, 91.

(3) Goodwin, J. W.; Russel, W. B. *Curr. Opin. Colloid Interface Sci.* **1997**, *2*, 409 and references therein.

(4) Hemar, Y.; Horne D. C. *Langmuir* **2000**, *16*, 3050.

(5) Princen, H. M. *J. Colloid Interface Sci.* **1983**, *91*, 160.

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tension and a being the drop radius). Moreover, the scaling with σ/a confirms that the elasticity of compressed monodisperse emulsions depends only on the packing geometry of the droplets.[1,5,6]

The earliest theoretical considerations[5−8] concerning $\Pi(\phi)$ and $G(\phi)$ of concentrated emulsions and foams were based on perfectly ordered crystals of droplets. In such systems, at a given volume fraction and applied shear strain, all droplets are supposed to be compressed equally, i.e., to deform affinely under the applied shear; thus, all of them should obey the same shape. Even when the scaling is predicted with the capillary pressure, these models[5−7] poorly agree with the experimentally found dependence of G' on the oil volume fraction. Lacasse et al.[9] performed Monte Carlo simulations aimed to predict the dependence of the elastic shear modulus on the volume fraction. Their simulations have two particularities which need to be emphasized. (i) The energy of deformation per facet formed between two neighboring droplets has been taken to be softer than harmonic. This permitted one to incorporate in the model the possibility of coupling between the different facets on one and the same droplet. (ii) The real microscopic structure of concentrated emulsions has been taken into account. Instead of imposition of a kind of crystal-like lattice, the model deals with systems of disordered droplets. The simulations provide a physical insight on the origin of the behavior of the shear modulus of emulsions. The unharmonicity of the potential has profound consequences on the deformation: even under a uniform compression, it turns out that there are nonaffine particle displacements. Indeed, it is shown that shear produces a positional relaxation (displacement) of the droplets and that the droplet motion is clearly nonaffine but is random in direction. The importance of this random motion is reinforced by calculating the shear modulus of a strictly affine (unrelaxed) motion; the elastic modulus increases by a factor of about 3 for all ϕ.

The aim of the experiments reported in the present paper was to provide a quantitative insight on the bulk elasticity properties of protein-stabilized concentrated emulsions. We prepared concentrated quasi-monodisperse hexadecane-in-water emulsions stabilized by various proteins, and we investigated their rheological properties. Some protein-stabilized emulsions possess remarkably high elasticity, and at the same time they are considerably fragile—they exhibit coalescence at yield strain and practically do not flow. The elastic storage modulus G' and the loss modulus G'' of the emulsions were determined for different oil volume fractions above the random close packing. Surprisingly, the dimensionless elastic modulus $G'/(\sigma/a)$ obtained for emulsions stabilized by different proteins do not collapse on a single master curve, being almost always substantially higher than the corresponding values obtained for equivalent SDS-stabilized emulsions. We propose some possible explanations for this unusual elastic behavior.

2. Experimental Section

2.1. Substances. The proteins employed in this study were bovine serum albumin (BSA; Acros product, fraction V, 96−100% protein on a dry basis), casein (Sigma product, C 0536, lot 14H9606), β-lactoglobulin (BLG; Sigma product, L-0130, lot 124H7045), lyzozyme (Sigma product, L-6876, lot 57H7045), and ovalbumin (Sigma product, A-5503, lot 108H7018). The proteins were used as received. The oil phase in all cases was hexadecane (Acros product, p.a. grade). In all samples the aqueous phase contained 0.001 M NaN_3 in order to suppress the bacterial growth. This salt fixes also the ionic strength. All solutions were prepared using water purified by a Milli-Q unit (Millipore), with a resistance of 18.2 MΩ·cm^{-1}. The pH in all cases was the natural one (see below), i.e., 5.7 ± 0.1 for BSA solutions, 6.1 ± 0.1 for casein solutions, 6.1 ± 0.1 for BLG solutions, and 7.2 ± 0.1 for lyzozyme. All experiments were carried out at room temperature, 22 ± 1 °C.

2.2. Methods. *pH control*. The pH values were measured by means of a digital pH meter, equipped with protein and a surfactant-resistant electrode. The proteins obey buffering properties. When dissolved in water, they generally maintain a certain pH value (which may depend on the purification process), frequently referred as "natural pH". This property of the proteins was used in our experiments to control the pH. For each protein (β-casein, BSA, BLG, lyzozyme, and ovalbumin), we verified that the pH is constant within 0.2 units over the range of concentrations studied. No drift of the pH values of the protein solutions was observed over the period of storage.

Measurements of the interfacial tension were performed on a Krüss tensiometer, using the du Nouy ring method. The measurements were performed on protein solutions of concentration 0.2 wt %, ionic strength 0.001 M (maintained also with NaN_3), natural pH, and room temperature 22 °C. The values were taken 24 h after loading the two liquid phases into the measuring vessel. According to the literature data,[10−12] this time is sufficient for reaching equilibrium between the bulk and the interface. In a parallel experiment, we checked that in each case a constant value of the interfacial tension is reached for even shorter times, say 12−14 h. The measured interfacial tension values were as follows: 14.5 mN/m for casein, 17.3 mN/m for BSA, 15.9 mN/m for BLG, 18.1 mN/m for lyzozyme, and 22.2 mN/m for ovalbumin. The values obtained are consistent with the data of other authors (see refs 10−12) obtained using different methods for the determination of the interfacial tension.

Emulsion Preparation. A primary crude emulsion at an oil volume fraction of 0.3−0.5 was prepared by vigorous shaking. The initial concentration of the protein solutions was typically between 1 and 3 wt % depending on the volume fraction of the premix. This crude emulsion was further homogenized in order to make it quasi-monodisperse. This was achieved by passing the premix a few times (at least six) through a two-chamber high-pressure homogenizer JH-1 (Lab Plant, England). Emulsions obtained in that way possess a narrow size distribution. Their mean size (diameter) was reproducible and was in all cases about 0.5 μm. By using well-controlled emulsions, consisting of droplets of approximately the same size, our study offers a few advantages in comparison with the previous rheological experiments,[4] which have been performed on emulsions having a broad size distribution. Indeed, very polydisperse emulsions are difficult to study, because they contain droplets of different Laplace pressure; hence, at a fixed osmotic pressure, the larger droplets may deform significantly, while the smaller remain essentially nondeformed. Moreover, for very polydisperse droplets the packing is much more efficient; hence, the evaluation of ϕ^* is not evident.

Once produced, our samples were further concentrated by centrifugation. The centrifuge employed was Sigma K300, rotor 12150. Generally, the emulsions were centrifuged for 30 min at 20 000 rpm. After centrifugation, the concentrated emulsion (cream) was carefully separated from the supernatant. The resulting concentrated emulsions were of oil volume fractions of about 0.9. Emulsions at lower oil volume fraction were obtained

(6) Princen, H. M.; Kiss, A. D. *J. Colloid Interface Sci.* **1986**, *112*, 427.
(7) Buzza, D. M. A.; Cates, M. E. *Langmuir* **1994**, *10*, 4503.
(8) Buzza, D. M. A.; Lu, C.-Y. D.; Cates, M. E. *J Phys. Fr. II* **1995**, *5*, 37.
(9) Lacasse, M.-D.; Grest, G. S.; Levine, D.; Mason, T.; Weitz, D. A. *Phys. Rev. Lett.* **1996**, *76*, 3448.
(10) Lucassen-Reynders, E.; Benjamins, J. In *Food Emulsions and Foams Interfaces, Interactions and Stability*; Dickinson, E., Rodríguez Patino, J. M., Eds.; Royal Society of Chemistry: London, 1999; p 195.
(11) Graham, D. E.; Phillips, M. C. *J. Colloid Interface Sci.* **1979**, *70*, 403, 415, 427.
(12) Makievski, A. V.; Miller, R.; Fainerman, V. B.; Krägel, J.; Wüstnek, R. In *Food Emulsions and Foams Interfaces, Interactions and Stability*; Dickinson, E., Rodríguez Patino, J. M., Eds.; Royal Society of Chemistry: London, 1999; p 269.

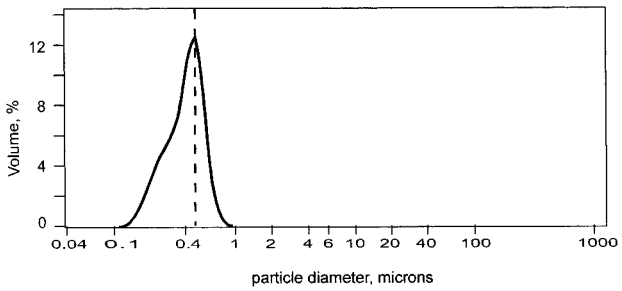

Figure 1. Typical droplet size distribution (by volume) of a hexadecane-in-water emulsion used in the rheological experiments. In this case the stabilizing protein is BSA (see the text for details).

by diluting the cream with a controlled quantity of the supernatant. After the supernatant was added, the samples were left for equilibration for at least 12 h. An alternative way for obtaining emulsions at lower volume fraction is a centrifugation either at lower acceleration or for a shorter period of time. After the centrifugation, the emulsions were examined by optical microscopy (Zeiss, Axiovert 100, objective 100×/1.3) and their droplet size distribution was measured. Both experiments showed that the droplets do not coalesce during the centrifugation.

Emulsion Characterization. The droplet size distributions of the emulsions were measured on a Coulter Counter LS230 static light scattering apparatus. The data acquisition was 90 s long, and at least three runs were made in each case. It was necessary to spend some effort in order to ensure that only *individual* droplets are present in the measuring cell. Prior to measurements, the emulsions were diluted 100 times with a 5 × cmc solution of Tween 20 (1 cmc ≈ 3×10^{-5} M). The measuring cell of the apparatus was filled with the same surfactant solution. It was necessary to dilute the emulsions with a surfactant solution in order to diminish the flocculation.[13] A typical size distribution of the emulsions used in this study is shown in Figure 1.

The volume fraction of the oil phase was determined in the following way. A part of each emulsion sample was left in a dry place at room temperature for approximately 12 h. This time is sufficient for complete evaporation of the aqueous phase. We verified that for the same period of time the volatility of hexadecane is negligible. Therefore, weighing the samples before and after the water evaporation provides a direct result for the oil volume fraction. The (small) amount of the protein left in the dried material was included in the calculation. Let us mention that the evaporation of the water always led to the formation of a completely *transparent solidlike material*, i.e., a perfect *biliquid foam*. To have better control of the evaporation, the samples were weighted during evaporation, and we can affirm that transparent samples contain more than 99% of the dried substance (oil + protein). Furthermore, the sample weight remained practically constant over several days. The latter gives another convincing proof that almost all of the water content was evaporated. In the case of a very low water content, as here, the droplets are polyhedral and the radius of curvature of the Gibbs−Plateau borders becomes very small.

We tried to determine the quantity of the protein left in the bulk aqueous phase, employing the two-wavelength photometry method by Warburg and Hristian.[14] The experiments showed that in all cases the free protein left in the continuous phase is about the detection limit of the method, i.e., 0.01−0.02 wt %. We had no access to an alternative method to determine the unabsorbed protein content in the continuous phase as was done, for example, by Dickinson et al.[15] However, the result described above gave us a hint that a reasonable estimation of the surface coverage can be made by assuming that the protein was totally adsorbed at the interface. This is a reasonable assumption, because it is also admitted that the proteins adsorb irreversibly at the oil/water interfaces.[16] Even being unable to cite an exact value, we are convinced that the bulk protein concentration is about or less than 0.02 wt %, which permits us to estimate the surface coverage with reasonable precision.

Osmotic Pressure Measurements. To measure the evolution of the osmotic pressure, we centrifuged a known amount of dilute emulsion and then determined ϕ by removing a small amount of the cream at the top of the centrifugation tube, followed by water evaporation. After creaming, if the droplets occupy a distance much less than that of the centrifuge lever arm, the spatial gradient in the acceleration can be neglected, and the osmotic pressure can be determined as[1,9]

$$\Pi = l\Delta\rho\omega^2\phi_i d_\perp \tag{1}$$

where l is the total liquid height in the centrifuge tube, ϕ_i is the initial oil volume fraction before the centrifugation, $\Delta\rho \approx 0.223$ g/cm^3 is the density mismatch between oil and water, $d_\perp$ is the length of the lever arm, and ω is the rotation speed of the centrifuge. This maximum osmotic pressure reflects the buoyant stress of the droplet layers below the top of the cream. The centrifugation typically takes several hours in order for an equilibrium volume fraction to be achieved. The centrifuge employed was an Optima TLX ultracentrifuge (Beckman) equipped with a swinging-bucket rotor, model TLS-55. Let us stress again the fact that the centrifugation did not lead to coalescence of the samples.

Measurements of the elastic and dissipation moduli of the concentrated emulsions were carried out on a controlled strain and stress rheometer CarriMed 500, equipped with different cone-plane measuring tools. In a typical measurement, a sinusoidal strain $\gamma_0(\omega)$ is applied at a given frequency, ω. The response of the system [i.e., the time-dependent stress, $\tau_0(\omega)$] is measured. For the linear regime (i.e., a perturbative sinusoidal strain is applied), the response of the studied material is also sinusoidal and shifted by a phase angle δ. The elastic and plastic moduli (G' and G'', respectively) are calculated as

$$G' = \frac{\tau_0(\omega)}{\gamma_0}\cos\delta(\omega)$$

$$G'' = \frac{\tau_0(\omega)}{\gamma_0}\sin\delta(\omega) \tag{2}$$

Above a certain (high) value of the applied strain, the deformation is no more perturbative, i.e., the response of the material is no more sinusoidal; therefore, eq 2 does not hold. The material begins to *flow*. Hence, in this case, G' and G'' should be considered only as apparent. For a complete review of the oscillatory rheology, the reader is directed to the book of J. D. Ferry.[17] We used a solvent trap in order to avoid water evaporation from the samples during the measurements. We checked that the values of G' and G'' are the same (in the frame of the experimental uncertainty) when one and the same sample is measured by employing two cones of different radii. We verified also that the volume fraction of each emulsion remains constant within 0.5% before and after the measurements. Each sample was measured at least 12 h after its preparation. This protocol was adopted in order to ensure a complete equilibration of the emulsion.

Thin liquid films were investigated in a Scheludko-type cell. The setup employed in these experiments is characterized extensively in the literature;[18] here we outline only the main points. The film is formed by sucking the liquid from a biconcave meniscus formed in a capillary of millimeter size. The latter is attached on the table of a light microscope (Jenavert, Carl Zeiss, Jena), and the films were observed in reflected illumination. The capillary pressure of the films is maintained by a system consisting of two syringes (coarse and fine manipulation) and a

(13) Dickinson, E.; Williams, A. *Colloids Surf.* **1994**, *88*, 317.
(14) Skopes, R. A. In *Metodyi Otchistki Belkov*; Mir Publishing House: Moscow, 1986; p 300 (in Russian).
(15) Dickinson, E.; Golding, M.; Povey, M. J. W. *J. Colloid Interface Sci.* **1997**, *185*, 515.
(16) Dalgleish, D. G. In *Food Emulsions and Foams Interfaces, Interactions and Stability*; Dickinson, E., Rodríguez Patino, J. M., Eds.; Royal Society of Chemistry: London, 1999; p 6 and references therein.
(17) Ferry, J. D. *Viscoelastic Peoperties of Polymers*; John Wiley and Sons: New York, 1980.
(18) Scheludko, A. *Kolloid-Z.* **1957**, *155*, 39.

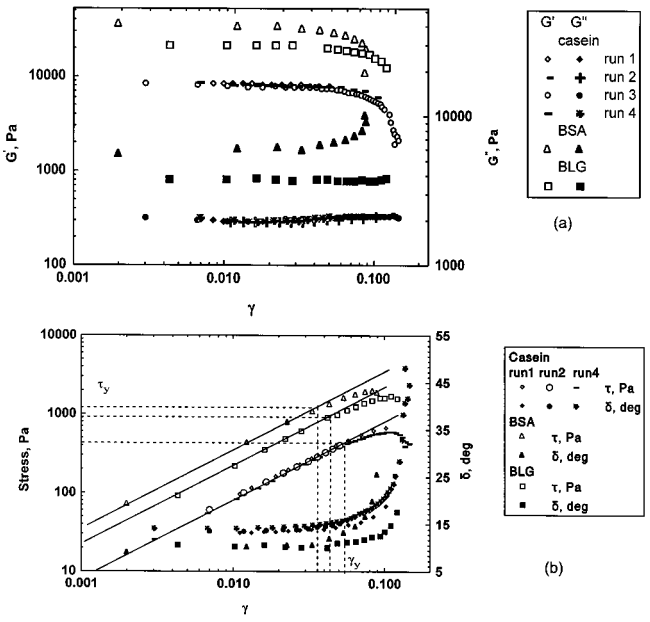

Figure 2. (a) Dependence of the elastic (G') and loss (G'') moduli on the applied strain for hexadecane-in-water emulsions, stabilized with casein, BSA, and BLG. In all cases the surface coverage is 2 mg/m^2, and the oil volume fraction is 0.725 for casein, 0.733 for BSA, and 0.717 for BLG. (b) Dependence of the stress (left axis), and phase angle (right axis) on the applied strain for the same emulsion as that in part a.

bulk of buffering liquid. This configuration provides the possibility of determining the contact angles of the films via light interferometry.[18,19] The contact angles were numerically calculated (with a precision of 0.1°) by adopting the scheme of Dimitrov et al.[19]

To mimic the real conditions under which the emulsions are prepared, i.e., the rapid distortion and numerous ruptures of the interface, in each case we formed several consecutive films. Each of them was subjected to a vigorous rupture, achieved through blowing a bubble inside the film.

3. Experimental Results

3.1. Crude Data. We examined the dependence of G' and G'' on the applied strain at different oil volume fractions and protein surface coverage for hexadecane-in-water emulsions stabilized by various proteins. Figure 2 shows four consecutive measurements (at frequency of 0.3 Hz) of G' and G'' on one and the same casein-stabilized sample. The oil volume fraction is 0.725, and the surface coverage is 2 mg/m^2. This set of data demonstrates the reproducibility of the measurements taken on one and the same sample. Data for equivalent emulsions stabilized with BSA (oil volume fraction of 0.733 and surface coverage of 2 mg/m^2) and BLG (oil volume fraction of 0.717 and surface coverage of 2 mg/m^2) are given for comparison. The shape of all curves present in Figure 2a is very typical for all of the protein-stabilized emulsions studied. It is also qualitatively similar to the data reported in the literature.[1] Figure 2b shows the evolution of the measured stress and phase shift (the angle δ). One can see that for strains smaller than ca. 0.056 for casein, ca. 0.042 for BLG, and ca. 0.037 for BSA the dependence of the stress on the strain in the log−log plot is linear and the slope equals unity. In other words, there is a plateau value in G' for strains lower than the cited values. The deformation and the corresponding stress at which the slope deviates from unity (in a log−log plot) are frequently designated as *yield strain* (γ_y) and *yield stress* (τ_y), respectively. Above the yield strain/stress, one switches from the perturbative regime to the regime where the microscopic structure of the emulsion is affected. In G' vs strain plot, the yielding point corresponds to the end of the plateau in G'. Evidently, at this point, the applied strain affects considerably the microscopic structure of the investigated sample. At strains larger than γ_y the measured stress is no more sinusoidal and the values for G' and G'' should be considered as apparent, because the material begins to flow. The above analysis has been applied for emulsions stabilized with SDS[1,9] (where the repulsion between the droplet interfaces is considerable) and the dependence of the yield stress on the volume fraction has been determined. In our case, when the microscopic structure is gradually affected, the emulsions coalesce instead of flowing. Here arises the question of whether it is possible to define yield stress and strain in the same manner as is done in ref 1, for example.

It is to be mentioned that the shear-induced coalescence was variably pronounced. In the majority of cases, some oil was released, with the latter being macroscopically detected. In some other cases, coalescence was not visible by the naked eye, but inspection under a microscope revealed the appearance of large oil droplets. In a recent study,[20] it was demonstrated that concentrated protein-stabilized emulsions exhibit a flow-induced coalescence. According to the authors, the flow-induced coalescence frequently follows a "fracture type of mechanism which involves the coalescence of many emulsion droplets along a slip or fracture plain". This is what we believe to happen for some of the systems, when oil release was observed. In other words, when a sufficiently high strain is applied, the emulsions coalesce.

Another important property of our systems is that no reliable measurement is possible when the oil volume fraction is very high, say above 0.9. Such emulsions are easy to be produced by centrifugation, but they coalesce during the loading/compression of the samples in the measuring cell, even if the compression is made at the lowest possible rate. As a limiting case, it was not possible at all to study the most concentrated emulsions (i.e., the biliquid foams we produced; see below) by means of the oscillatory rheology, because they immediately coalesced upon application of extremely low shear. This qualitative observation is in agreement with the work of van Aken and Zoet,[20] who also pointed out that the flow-induced coalescence increases with the volume fraction. Evidently, an alternative method for the determination of G' and G'' should be sought for the highest oil volume fractions. We should admit that the loading of the samples in the measuring cell is intrinsically connected with some shear which may provoke some coalescence of the emulsion. This is, in our opinion, the reason our data are somehow more scattered than usual. For the same reason, we did try to quantify the shear-induced coalescence in the samples; even Figure 2 suggests that the nature of the protein has an influence on the "yield" stress/strain. We took all possible precautions in order to diminish the "preshear" when loading the samples: the emulsions were placed in the cell of the rheometer very carefully; the sample compression during loading was the minimal possible and was always done at the smallest rate.

Coming back to the measured moduli, we have to stress the fact that the loss modulus G'' is more than 1 decade lower in value than G' (Figure 2). This result reflects the essentially elastic nature of the concentrated emulsions.

(19) Dimitrov, D.; Kralchevsky, P. A.; Nikolov, A. D.; Wasan, D. T. *Colloids Surf.* **1990**, *47*, 299.

(20) van Aken, G. A.; Zoet, F. D. *Langmuir* **2000**, *16*, 7131.

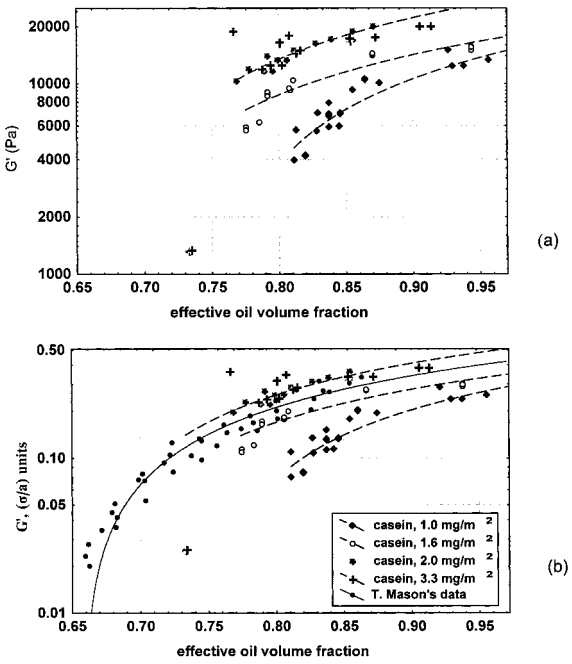

Figure 3. Storage modulus (G') (measured at a frequency of 0.3 Hz) of hexadecane-in-water emulsions, stabilized by casein, as a function of the effective oil volume fraction: (a) absolute scale; (b) the same set of data as that in part a but with G' normalized by σ/a. In all cases, the lines are guides for the eye.

This is why in the following sections we shall comment only on the evolution of G'.

To summarize, let us emphasize that our emulsions practically do not flow. Instead, they exhibit shear-induced coalescence, with the latter being enhanced with the oil volume fraction. The absence of plastic flow is a substantial difference in comparison with the emulsions stabilized with classical surfactants.[1] At this point, we should comment that our results are somehow in contradiction with a recently published study, dealing with concentrated emulsions stabilized by sodium caseinate.[4] The emulsions reported in ref 4 exhibited plastic flow, which is not the case in our experiments. We believe that the broad distribution of the samples in ref 4 shifts the random close-pacing limit to considerably higher volume fractions, thus reducing the effective deformation of the droplets.

3.2. Casein. In the case of casein-stabilized emulsions, we performed a set of experiments in which we varied the protein surface coverage of the emulsion droplets (Figure 3a). The measurements were taken at a constant frequency of 0.3 Hz. The graph shows the dependence of G' as a function of the effective oil volume fraction for different protein surface coverages. Hereafter, the commented values of G' refer to the plateau value in the strain−G' curves. The frequency sweep (in a linear regime) showed no particular differences in comparison with the previous studies.[1] It appears that increasing the surface coverage up to its value at saturation (about 2 mg/m^2) leads to an increase in the magnitude of the elastic modulus. Once a relatively dense coverage is achieved, the elastic modulus seems not to change any more. Anyway, we should admit that the differences, even detectable, are not substantial.

We observed that the measured values of G' are considerably higher than the elastic moduli of equivalent emulsions stabilized with SDS[1] (Figure 3a). However, it is more instructive to plot the data in the $G'/(\sigma/a)-\phi_{\text{eff}}$ coordinates, with ϕ_{eff} being the effective oil volume fraction. This type of plot provides the possibility of comparing the results with the literature data excluding the effects of particle size and interfacial tension. The effective oil volume fraction may be evaluated by taking into account the repulsive interactions between the droplets. Actually, as pointed out by Princen,[5,6] the effective oil volume fraction takes into account the thin films which exist between the deformed droplets. It is important to note that the droplets start to deform before the surfaces actually touch. The effective oil volume fraction can be calculated as[9]

$$\phi_{\text{eff}} \approx \phi\left[1 + \frac{3}{2}\frac{h(\phi)}{a}\right] \tag{3a}$$

where $h(\phi)$ is the thickness of the films between the droplets at volume fraction ϕ. The dependence $h(\phi)$ is not known; hence, we are obliged to adopt an approximation. Following ref 1, we can consider that h is a linear function of ϕ. Hence, one needs to evaluate the film thickness at two different oil volume fractions (above the random close packing). It is reasonable to suppose that, for $\phi_{\text{eff}} \approx \phi^* = 0.64$ (noncompressed droplets), the film thickness $h_{\max}$ is equal to the equilibrium film thickness which is measured in different experiments[21,22] designed to determine the force vs distance laws existing between emulsion droplets. We take $h_{\max}$ to be 20 nm.[21,22] Because we are able to produce perfectly transparent *stable* biliquid foams by free evaporation of water, we can consider that the protein films are essentially free of water. These biliquid foams are composed of polyhedral droplets surrounded by protein films. Evidently in this case we have $\phi_{\text{eff}} \approx 1 = \phi_{\text{dried}} + \phi_{\text{protein}}$. The quantity of the protein ϕ_{protein} in the biliquid foam can be easily calculated from the initial protein content in the emulsion. Therefore, one can calculate the film thickness, $h_{\min}$, as

$$h_{\min} \approx \frac{2a}{3}\left(\frac{1}{\phi_{\text{dried}}} - 1\right) \tag{3b}$$

where ϕ_{dried} is the fraction of the oil in the biliquid foam. (Generally, ϕ_{dried} was in the limit 0.96−0.99.) For casein, we find $h_{\min} = 3.5$ nm (for $\phi_{\text{dried}} \approx 0.980$). As a side comment, let us mention that the value which we obtained through eq 3a coincides very well with twice the thickness of *dense* protein adsorbed layers, measured by neutron reflectivity.[23] Atkinson et al.[23] have found that adsorbed β-casein (the major constituent of whole casein) forms a layer which is about 1.5 nm thick and the volume fraction of the protein in it is about 90%.

Now it is possible to transform ϕ into ϕ_{eff} and to compare our data with those cited in ref 1. As one can see, our results collapse reasonably well to the master curve, obtained by Mason et al.[1] (Figure 3b). Even narrow-sized, our emulsions are not as monodisperse as those studied in refs 1 and 9. This is the reason in some cases why we found slightly smaller dimensionless values for the elasticity. For a more detailed study about the influence of the polydispersity on the rheological properties of emulsions, the reader is directed to ref 1.

3.3. Lyzozyme and BLG. To investigate the role of the type of protein on the rheological properties of emulsions, we performed the same experiments on emulsions stabilized with lyzozyme and BLG. The surface coverage is estimated to be 2 mg/m^2 in both cases, and the measuring frequency was 0.3 Hz. The results are sum-

(21) Dimitrova, T. D. Ph.D. Thesis, University of Bordeaux, Bordeaux, France, 2000.
(22) Dimitrova, T. D.; Leal-Calderon, F. *Langmuir* **1999**, *15*, 8813.
(23) Atkinson, P. J.; Dickinson, E.; Horne, D. S.; Richardson, R. M. *J. Chem. Soc., Faraday Trans.* **1995**, *91* (17), 2847.

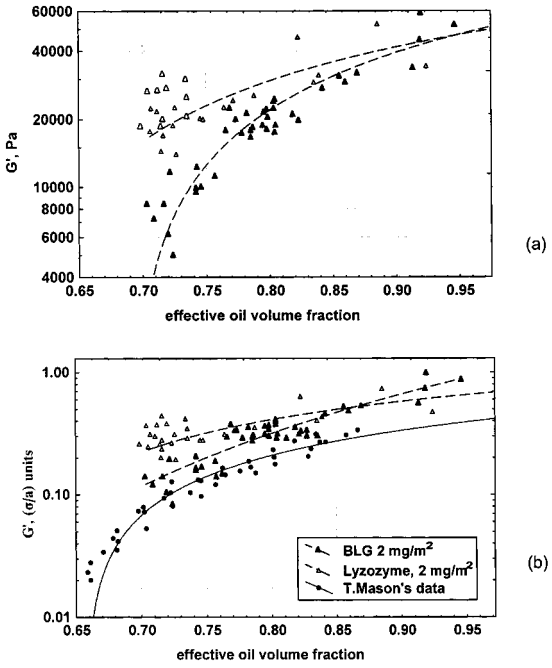

Figure 4. Storage modulus (G') (measured at a frequency of 0.3 Hz) of hexadecane-in-water emulsions, stabilized by lyzozyme and BLG as a function of the effective oil volume fraction: (a) absolute scale; (b) the same set of data as that in part a but with G' normalized by σ/a. The lines are guides for the eye.

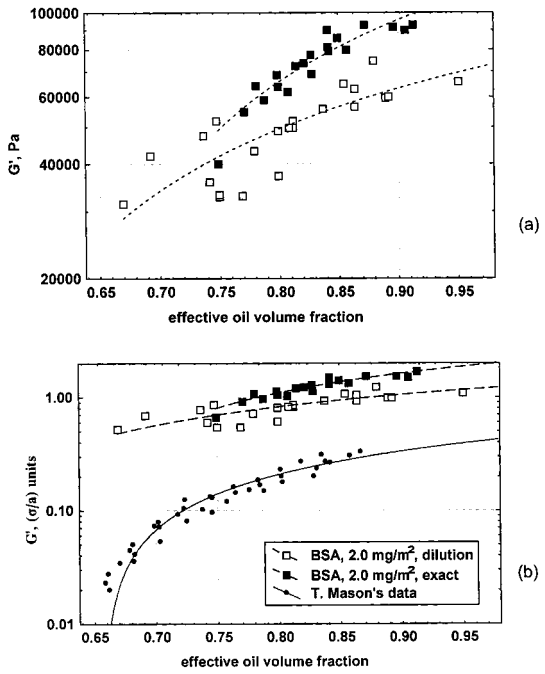

Figure 5. Storage modulus (G') (measured at a frequency of 0.3 Hz) of hexadecane-in-water emulsions, stabilized by BSA, as a function of the effective oil volume fraction: (a) absolute scale; (b) the same set of data as that in part a but with G' normalized by σ/a. The lines are guides for the eye. The notations "dilution" and "exact" refer to the manner of preparation (see the text for details).

marized in Figure 4. As in the previous case, the elasticity was normalized by the capillary pressure and compared with the results obtained in ref 1. In both cases, we transformed the oil volume fraction in the effective one as explained above. For BLG we took $h_{max} = 16$ nm (the closest surface-to-surface distance resolved in magnetic chaining technique experiments[21]) and $h_{min} = 4$ nm (for $\phi_{dried} \approx 0.979$). For lyzosyme we took $h_{max} = 12$ nm (data obtained on foam films[24]) and $h_{min} = 4.2$ nm (for $\phi_{dried} \approx 0.976$). As in the previous case, the calculated film thickness is approximately twice the thickness of dense individual protein layers (see ref 23).

As is evident from Figure 4, our results for a dimensionless elastic modulus in both cases do not collapse with the master curve, being at least 2 times higher. These samples were also very fragile and coalesced above a critical yield strain (Figure 2).

3.4. BSA. Figure 5 shows the dependence of the plateau value of G' as a function of the oil volume fraction in the case of BSA-stabilized hexadecane-in-water emulsions. The protein surface coverage in this case is about 2 mg/m^2. The frequency was, as in the previous cases, 0.3 Hz. One can see that the obtained values of G' are several times higher than those measured in the case of the samples stabilized by the other three proteins. We normalized ϕ by taking $h_{max} = 8$ nm (see ref 22) and $h_{min} = 3.9$ nm (for $\phi_{dried} \approx 0.982$). The normalized values are approximately half a decade higher than those obtained on equivalent emulsions stabilized with SDS (Figure 5b). Further comparison of casein- and BSA-stabilized emulsions (Figure 2) shows that the "yield" strain in the case of BSA is considerably lower than the one exhibited by casein-stabilized samples. To summarize, the BSA-stabilized emulsions are much more elastic than the casein-, lyzozyme-, and BLG-stabilized ones, but at the same time they coalesce more easily at relatively lower deformation.

In the case of BSA, we observed another interesting phenomenon. The overall rheological behavior of BSA-stabilized emulsions is dependent on the history of the sample preparation. In Figure 5, the notation "diluted" refers to the case where the emulsion is first centrifuged and then carefully swelled with a corresponding quantity of the supernatant. The notation "exact" corresponds to the case where the final oil volume fraction is achieved by appropriate centrifugation only. Note that the droplet mean size and the age of the samples are the same in both cases. Up until now, we were not able to propose an explanation concerning this effect. Various literature data[25−27] indicate the influence of history of the BSA-stabilized samples on their behavior, but a direct correlation of these results with our data is difficult to establish.

3.5. Ovalbumin. The samples stabilized by this protein exhibited a striking rigidity similar or even higher than that for BSA-stabilized samples. This unfortunately obstructed the reliable measurements of the elastic modulus, because even at volume fractions of about 0.7−0.8 the emulsions considerably coalesce during the sample loading in the measuring cell.

3.6. Osmotic Pressure Measurements. We determined the osmotic equation of state of the emulsions stabilized by BSA and casein. The results were normalized by σ/a and coplotted with the results for an emulsion stabilized by SDS (Figure 6). As can be seen from the graph, the difference between the three cases is not very important, keeping in mind the experimental uncertainty as well as the fact that the range of volume fraction which

(24) Marinova, K. G. To be published.

(25) Fitzpatrick, H.; Luckham, P. F.; Eriksen, S.; Hammond, K. *Colloids Surf.* **1992**, *65*, 43.

(26) Marinova, K. G.; Gurkov, T. D.; Velev, O. D.; Ivanov, I. B.; Campbell, B.; Borwankar, R. P. *Colloids Surf.* **1997**, *123−124*, 155.

(27) Gurkov, T. G.; Marinova, K. G.; Zdravkov, A. Z.; Oleksiak, C.; Campbell, B. *Prog. Colloid Polym. Sci.* **1998**, *110*, 263.

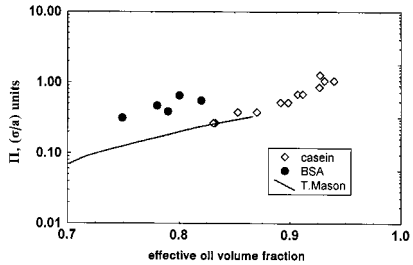

Figure 6. Osmotic pressure as a function of the oil volume fraction for hexadecane-in-water emulsions, stabilized by BSA or casein. The data are normalized with σ/a. The solid line is the result in ref 1 for SDS-stabilized silicone oil-in-water emulsions.

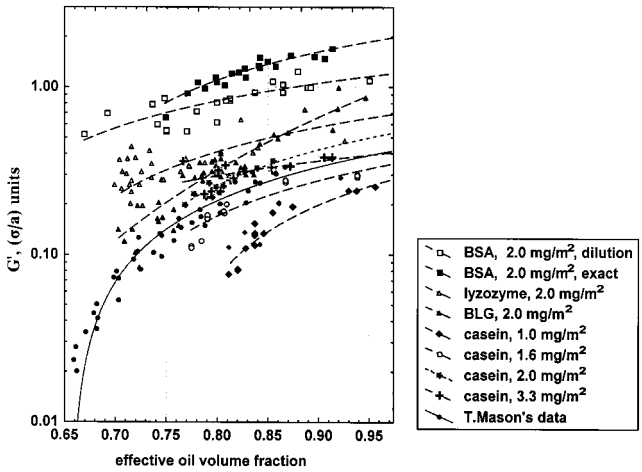

Figure 7. Elasticity of hexadecane-in-water emulsions, stabilized with different proteins, as a function of the effective oil volume fraction. The data are normalized with σ/a. The lines are guides for the eye.

has been explored is quite narrow. Actually, the osmotic pressure experiment corresponds to compression (deformation) of the droplets at zero frequency. From Figure 6, we deduce that, unlike the elastic modulus, the osmotic pressure does not reveal any specificity of the protein-covered interfaces, compared to surfactant-covered interfaces. This result suggests that the peculiar elasticity of the protein-stabilized samples cannot be attributed to any specific properties of the bulk continuous phase. Furthermore, the continuous phase was in all cases a Newtonian liquid. Let us stress that the experimental points in our case refer to a $\Pi(\phi)$ curve which is *only* valid for compression. Indeed, compressed emulsions did not redisperse, even being in contact with a large quantity of the corresponding aqueous phase for a very long period of time. We believe that the adsorbed proteins form a rigid interconnected network under compression which cannot be totally disjoined by the osmotic swelling.

4. Discussion

From the previous experiments and results, we learned that some protein-stabilized emulsions possess remarkably high elasticity in comparison with equivalent emulsions, stabilized by classical surfactants. All of our data for all studied systems are summarized in Figure 7, where we plot G' (in σ/a units) as a function of the oil volume fraction. The stronger effect is observed with BSA, followed by BLG and lyzozyme, and finally casein. The critical strain at which the samples coalesce increases in the same order (see Figure 2). However, the latter effect was difficult to accurately quantify. We aim now to propose some possible routes to explain the unusual elastic behavior.

We shall comment on two different factors that may influence the elasticity of the concentrated emulsions: (i) adhesion or stickiness of the protein-stabilized films and (ii) elasticity of the protein interfacial layers.

4.1. Adhesion or Stickiness in the Protein-Stabilized Emulsion Films. Useful information concerning the properties of protein-stabilized emulsions can be obtained from model experiments on thin liquid films. We performed experiments with different proteins in order to mimic the films present in concentrated emulsions. Let us first note that all of the proteins studied, especially BSA and BLG, were found to aggregate, forming visible clusters at the interface. Dynamic light scattering from bulk solutions of BSA and BLG revealed no clustering in bulk solutions, while large aggregates at the surface were clearly observed during thin liquid film studies.[21,27] The case of casein is slightly different, because this protein is a mixture of several individual proteins which strongly interact in the bulk solution. For this particular protein, we cannot state (as for BSA and BLG) that the clusters observed in the films are produced only by surface aggregation.

The formation of the films in a quasi-static manner was found to be a necessary prerequisite to inhibit surface aggregation, while the rapid distortion of a protein-covered interface always led to the appearance of aggregates.[21] Because our emulsions were prepared under non-quasi-static conditions (i.e., turbulent flow during emulsion preparation), we have a very strong reason to believe that surface aggregation occurred. When investigating thin films, we tried to simulate more or less the conditions for fabrication of real emulsions. In each case we produced numerous films, each of them being vigorously ruptured by powerful air flux and formed again. Under such conditions, we always observed an enhanced surface aggregation, as evidenced in Figure 8.

Generally, the investigated films possessed a nonzero contact angle (as calculated from the interference pattern), but in all cases the equilibrium contact angle did not exceed 1°. The energy of adhesion between the film surfaces is connected with the *advancing contact angle* as $W_{adh} = -2\sigma(1 - \cos\theta_{adv})$. The latter can be measured when one shrinks the film by decreasing the capillary pressure in the surrounding meniscus. In Figure 8 are shown a few consecutive steps in decreasing the capillary pressure in an emulsion film stabilized by BSA. The ionic strength was fixed by 0.001 M NaCl, the pH was the natural one, and the oil phase was hexadecane. When shrinking the films, we found that the advancing contact angle is practically equal to its equilibrium value, which corresponds to a low adhesion between the film surfaces. This is true for all emulsion films stabilized by each of the studied proteins. A more interesting phenomenon is connected with the *local stickiness* on the protein aggregates/lumps, which appears in some cases.[27] Generally, when the capillary pressure in the meniscus is decreased, the radius of the film also decreases until the surfaces are completely disjoined. If there is no local stickiness, the film remains circular during the process of shrinkage. In our experiments, the local bridging or stickiness between the surfaces occurs when surface aggregates are formed. The local stickiness obstructs the disjoining of the surfaces at the points of bridging, which results in the highly irregular (not circular) shape of the film. This effect is most strongly pronounced for the emulsion films stabilized by BSA. In Figure 8, the arrows indicate some of the points of sticking between the two surfaces of the film. In our opinion, this effect may influence the macroscopic rheo-

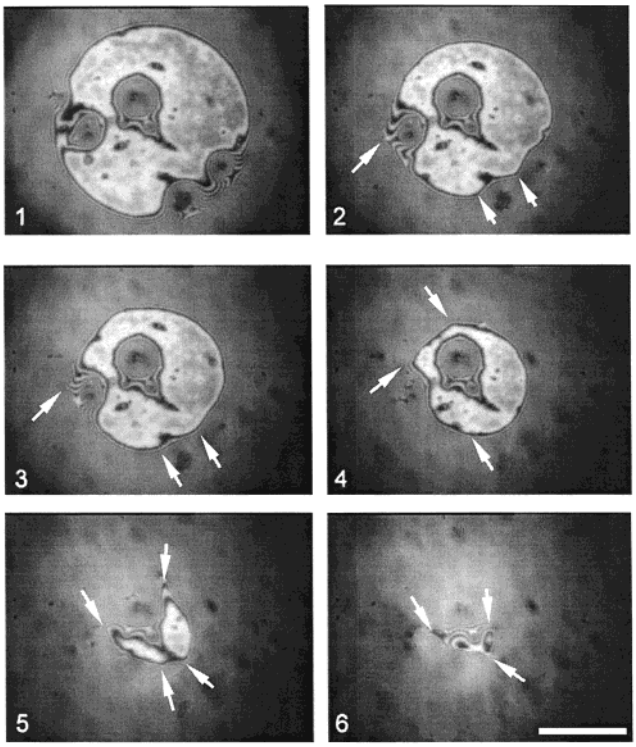

Figure 8. Six consecutive steps in shrinking of an emulsion film stabilized with 0.1 wt % BSA. The oil phase is hexadecane; the ionic strength is 0.001 M. The bar corresponds to 100 μm. The local adhesion on aggregates is evident. Arrows indicate some of the points of sticking at the interfaces. See the text for details.

logical behavior of concentrated BSA-stabilized emulsions. Indeed, it is natural to expect the same local stickiness to be present also in the films formed between the droplets in concentrated emulsions. As suggested in ref 9, the positional nonaffine displacements of the droplets upon a shear strain strongly affect the elastic modulus. From theoretical calculations the authors have found that the absence of such local rearrangements increases the shear modulus by a factor of 3. In our case, the local stickiness on the aggregates, especially in BSA-stabilized emulsions, obstructs the droplets' displacement under the application of a perturbative strain, which may produce an increase in the elastic modulus G'.

When the applied stain is no more perturbative, the droplets are supposed to be considerably displaced from their original positions which, on a macroscopic scale, means *flowing* of the material. In our case this cannot be realized because the displacement of the droplets from their relative positions requires a rapid breakage of the sticky contacts without rupturing the films themselves. The latter is not evident, and it is more realistic to expect that some of the films will be ruptured during the relative displacement of the emulsion droplets; i.e., coalescence will occur. Actually, such coalescence is believed to occur in our rheological experiments once a relatively high strain is applied.

Thus, in our opinion, the presence of sticky protein lumps/aggregates can be considered as one of the reasons for the high elasticity of the concentrated protein-stabilized emulsions, but these lumps make the samples very fragile also. The stickiness on the aggregates in the case of other proteins seems not to be as strong as that in the BSA case.

4.2. Elasticity of the Protein Layers. It is widely accepted[13] that proteins stabilize the emulsions, forming a viscoelastic film at the interface. A protein layer can be considered as a two-dimensional body, which has its own rheological properties.[10,11] The rheological properties can be defined for both dilatational and shear deformation. For an extensive review, the reader is directed to ref 10. Actually, in real situations, it is not evident to distinguish between the dilatational and shear effects. The elasticity of the protein layers is generally much higher than the elasticity of the low molecular weight surfactants, and because the protein layers can store energy themselves, it is reasonable to expect this effect to influence the bulk (three-dimensional) rheology of emulsions. As discussed by Buzza et al.,[8] the free energy change δF of a piece of interface whose area A is increased by δA obeys

$$\delta F/A = \sigma(\delta A/A) + (1/2)\epsilon(\delta A/A)^2 \qquad (4)$$

where ϵ is the elasticity of the layer, which can be formally defined in different ways.[8] For a static strain (zero frequency), one has $F \propto G\alpha^2$, where $G = G'(\omega=0) \propto \sigma/a$. This arises purely from the first term on the right-hand-side in eq 4. At nonzero frequency, the local variation of the interfacial tension will also contribute and the stored elastic energy can be higher than in equilibrium (zero frequency). Note that the term "nonzero frequency" should be considered always in conjunction with the characteristic relaxation time of the surfactant/protein layer stabilizing the emulsion. As demonstrated in ref 10, the viscoelasticity of dense protein layers is frequency-dependent, because of the surface relaxation processes. These include (i) diffusional interchange with the bulk, (ii) relaxation processes in the surface layer, such as, for example, reorientation and configurational changes in the proteins at the interface, surface "reactions", (iii) formation or destruction of three-dimensional structures at the surface or in the adjoining phase. The last two processes can obey a time scale ranging from 10^{-2} to 10^3 s. All of these phenomena are well documented in the literature for the case of protein-covered interfaces.[10,11,28,29]

Having in mind these remarks, we try now to find out some correlation between the dimensionless bulk elasticity of our emulsions and the surface elasticity of equivalent protein layers. In Table 1, we provide some literature data about the dilatational elasticity of the protein and SDS layers. We remark that the elasticity of the emulsions qualitatively varies in the same order as the elasticity of the respective isolated protein layers. Unfortunately, the available data for the elasticity of the

Table 1. Dilatational Moduli of Different Protein Layers (*Italics*, Triglyceride Oil/Water Interface)

protein (surfactant)	dilatational modulus, mN/m	
	air/water	oil (hydrocarbon)/water
(SDS)	<10[a]	<5[a]
β-casein	16.4,[b] 12.7[c]	*~8.5*[b]
κ-casein	20.6[b]	*~17*[b]
(whole) casein	≤20.0[b]	*~9*[b]
Na caseinate	20.6,[b] ~5[d]	*~3*[d]
lyzozyme	38.5[c]	
BLG	~29[d]	*~17*,[b] ~16[d]
BSA	~60,[b] ~40,[c] 65[e]	*~26*[b]

[a] Reference 30. [b] Reference 10. [c] Reference 11. [d] Reference 29. [e] Reference 28.

(28) Rodríguez-Patino, J. M.; Rodríguez-Niño, R. In *Food Emulsions and Foams Interfaces, Interactions and Stability*; Dickinson, E., Rodríguez Patino, J. M., Eds.; Royal Society of Chemistry: London, 1999; p 246.

(29) Murray, B. S.; Færgemand, M.; Trotereau, M.; Ventura, A. In *Food Emulsions and Foams Interfaces, Interactions and Stability*; Dickinson, E., Rodríguez Patino, J. M., Eds.; Royal Society of Chemistry: London, 1999; p 223.

proteins at the hydrocarbon/water interface are scarce, which is most probably because of the experimental difficulties in performing such measurements. It is clear that a straightforward comparison of the data from different sources is not always possible. First, the experiments on viscoelastic layers differ in the ionic strength, pH, etc., and, second, the experimental conditions (for example, the method employed, the rate of deformation, etc.) are not identical. This is why we only took those values for the dilatational moduli which have been determined for protein adsorption and deformation frequencies similar to those in our rheological experiments. As shown by Lucassen-Reynders,[10] the differences in pH and in the salt content lead generally to inferior differences. The correlation between the overall emulsion elasticity and the protein layer elastic modulus is more or less clear: in both cases elasticity goes like BSA > lyzozyme ≥ BLG > casein. In Table 1 we cite also data (for the sake of comparison) concerning the elasticity of SDS layers at air/water and dodecane/water interfaces. It is worth noting that these values are determined for the bulk concentration of SDS below the critical micellar concentration (cmc). Above cmc (i.e., saturated monolayers) the relaxation of the surface upon deformation is much more rapid; hence, the values of the elasticity will be even lower.[30] In other words, the surface elasticity effects in the presence of a low molecular weight surfactant will be undetectable at the typical frequencies applied by mechanic rheometers. We believe this is why this effect is neither detected nor considered in refs 1, 6, and 9.

Finally, it is worth noting that the two factors, i.e., the existence of sticky contacts and the high viscosity of the layer, cannot be considered separately, because both arise from the specific interactions between the adsorbed protein molecules and are intrinsically interrelated.

5. Biliquid Foams

As previously mentioned, we were able to produce easily extremely concentrated biliquid foams which are very stable at zero strain and cannot be redispersed spontaneously. Biliquid foams stabilized with different surfactants are reported in the literature,[31] but a necessary prerequisite for their stability is that they are to be kept in a place of controlled humidity. In other words, when exposed to the atmosphere, the biliquid foams based on low molecular weight surfactant degrade and release oil because the stabilizing surfactant layers are totally dehydrated. On the contrary, the biliquid foams in our case are produced by free evaporation of water. Thus, we managed to produce emulsions constituted by polyhedral droplets (Figure 9). Provided the bacterial growth is inhibited, the stability (the shelf-life) of these systems is considerably high, because of the resistance of the protein layers against rupture. The latter probably arises from the specific nature of the layers of denatured proteins that cover each droplet and which are sufficiently thick and rigid to protect the globules against coalescence.

Generally concentrated emulsions can be spontaneously redispersed, when put in contact with a large amount of the continuous phase for several hours. The reason is the osmotic swelling of the compressed material.[6,8,9] In contrast, our biliquid foams do not redisperse spontaneously, even after being in contact with the dispersed phase for days. Actually, there is some swelling of the structure

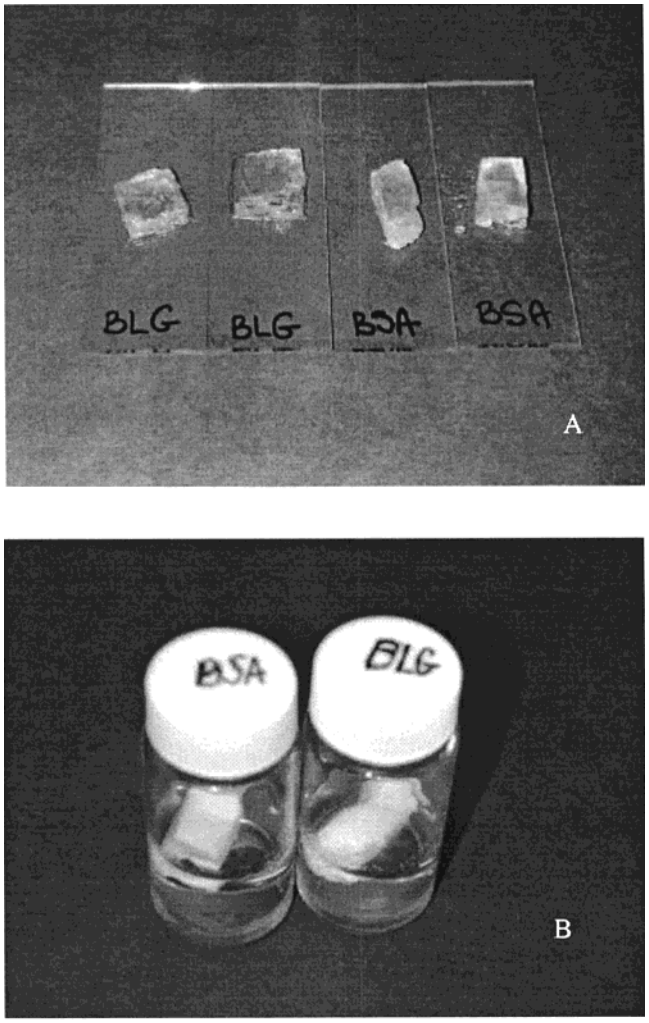

Figure 9. (A) Hexadecane-in-water biliquid foams obtained from concentrated emulsions by free evaporation of the aqueous phase at room temperature. The stabilizing protein is indicated on each slide. (B) The same samples as those in part a, being immersed for 48 in the appropriate continuous phase.

(it becomes turbid), as can be seen in Figure 9b, but no redispersion was observed for several days. We believe that the stabilizing protein forms a rigid network[32] on the surface of the fat globules, thus providing such "unusual" properties to the concentrated emulsions.

These biliquid foams obey striking properties. They are the kinds of solids which, on gentle touch, exhibit a strong elastic response. However, they are fragile: at strong deformation they release oil. As stated in section 3, this makes it impossible to evaluate their elastic modulus by employing a classical mechanical rheometer.

6. Conclusions

The aim of the experiments reported in the present paper was to study in a qualitative manner the fascinating elastic properties of concentrated protein-stabilized emulsions. To accomplish this goal, we prepared quasi-monodisperse hexadecane-in-water emulsions stabilized by various proteins of high purity. The properties of the films formed between the droplets were gleaned in experiments on thin emulsion films formed in a capillary cell. The shear elasticity modulus (G') of the samples was probed by oscillatory rheology, and the obtained data were

(30) Colin, A. Ph.D. Thesis, Université Paris VI, Paris, 1994.
(31) Sonneville-Aubrun, O.; Bergeron, V.; Gulik-Krzywicki, T.; Jonsson, B.; Wennerström, H.; Linder, P.; Cabane, B. *Langmuir* **2000**, *16*, 1569.
(32) Dalgleish, D. G. *Colloids Surf.* **1990**, *46*, 141.

compared with data from the literature obtained on monodisperse silicon oil−water emulsions stabilized by SDS.

Contrary to the surfactant-stabilized samples, protein-stabilized emulsions did not exhibit plastic flow but revealed shear-induced coalescence. In the linear regime, the elastic modulus G' was found to be dependent on the type of protein. For some proteins like BSA, lyzozyme, and BLG, the normalized elastic modulus $[G'/(\sigma/a)]$ is substantially larger than the one obtained for equivalent emulsions stabilized by low molecular weight surfactants. The samples stabilized by casein were less elastic and resemble classical surfactant-based emulsions.

We believe that the stickiness induced by surface protein aggregation is an important parameter because it may determine both the high elastic response of the concentrated emulsions and the coalescence in these samples when the latter are subjected to relatively high strain. On the other hand, at the measuring frequencies in our experiments, the dilatational elasticity of the protein layers is quite large and, as proposed in the literature, could have a substantial contribution to the total elasticity of the emulsions. The results reported in this paper could be considered as a first step in the investigation of concentrated globules covered by dense elastic layers.

Finally we observed that protein-stabilized concentrated emulsions can be dried at room temperature until a perfectly transparent biliquid foam is formed. Thus, we were able to obtain rigid materials whose existence is possible only because the stabilizing protein layers are sufficiently dense and rigid to maintain the cellular structure.

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