Surface Tension of Bovine Serum Albumin and Tween 20 at the Air–Aqueous Interface

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ABSTRACT: Interfacial properties (surface tension, σ, and critical micelle concentration, CMC) of aqueous solutions of Tween 20 (polyoxyethylene sorbitan monolaurate) and/or bovine serum albumin (BSA) were evaluated. Temperature, Tween 20 concentration in the aqueous phase, BSA/Tween 20 ratio, and aqueous phase composition [water, ethanol (0.5, 1.0, and 2.5 M), and sucrose (0.5 M)] were the variables studied. The CMC of Tween 20 was determined by surface tension measurements (Wilhelmy plate method). The existence of BSA–Tween 20 interactions was deduced from surface tension measurements. The results show that the effect of temperature on CMC depends on the aqueous phase composition, but the σ value at CMC, σ_{CMC}, does decrease as temperature is increased. The CMC and σ_{CMC} values also depend on the aqueous phase composition. In aqueous ethanol solutions, the CMC increases, but σ_{CMC} decreases. However, in sucrose aqueous solutions, the CMC decreases, but there is no significant effect on σ_{CMC}. The BSA–Tween 20 interactions at the interface depend on both Tween 20 concentration (C) and solute in the bulk phase. In water and aqueous solutions of ethanol and sucrose, σ values decrease in the presence of protein at $C <$ CMC but are practically independent of C at $C >$ CMC. This is an indication that the interfacial characteristics of the mixed film are determined by either the protein or the lipid at the higher and lower protein/lipid ratio, respectively. In the intermediate region, the existence of BSA–Tween 20 interactions dominates the interfacial characteristics of mixed films.

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KEY WORDS: Adsorption, air-water interface, bovine serum albumin, critical micelle concentration, food emulsifiers, protein-lipid interactions, surface tension, surfactant, Tween 20 (polyoxyethylene sorbitan monolaurate), tensiometry.

The formation of stable colloidal dispersions (emulsions, foams, etc.) is extremely important in the paint, petroleum, cosmetic, pharmaceutical, and food industries (1–3). Significant economic waste arises from the manufacture of unstable foams and emulsions. The chemical and physical properties of surface-active molecules are of great interest because they determine the colloidal stability of dispersed particles (4). Proteins stabilize foams and emulsions by forming a mechanical barrier at the interface that encapsulates the dispersed phase and resists random surface perturbations, droplet coalescence, or flocculation (5). The barrier is formed as intermolecular interactions between the adsorbed protein molecules are established. These contribute significantly to the rheological properties of the barrier and immobilize proteins in the adsorbed layer. In contrast, lipids stabilize the dispersed droplet or bubble by formation of a densely packed monomolecular layer, which is stabilized by Gibbs-Marangoni mechanisms (6). However, the presence of proteins and lipids in the same systems can result in instability as both types of surface-active molecules compete to form different types of adsorbed layers (immobile vs. mobile) (7). The distribution of proteins and lipids in food emulsions and foams is determined by competitive and cooperative adsorption between the two types of emulsifiers at the fluid–fluid interfaces, and by the nature of protein–lipid interactions, both at the interface and in the bulk phase (6,8). Thus, an understanding of these phenomena is a key factor in the development of strategies for controlling dispersion instability.

In the preceding paper (9), we studied the influence of solutes in subphase (ethanol and sucrose) on the adsorption isotherm of a protein (bovine serum albumin, BSA) and on the interactions between this protein and two insoluble lipids (monostearin and monoolein). The major conclusions were the following: the competitive adsorption of ethanol and BSA decreased both the surface activity and the monolayer saturation by the protein. However, the opposite was observed in the presence of sucrose in the bulk phase. The surface activity of BSA–lipid mixed films was determined by the component present in the highest concentration. Moreover, the existence of protein–lipid interactions depended on the protein/lipid ratio and on the aqueous phase composition. The existence of protein–lipid interactions can have a pronounced impact on the surface rheological properties of these systems (10–14). So, these results are of practical importance for food emulsion formulation, texture, and stability.

This work is an extension of studies of BSA adsorption from aqueous solutions that are of interest for food formulations. The aim of this paper is to study the effect of ethanol and sucrose in the subphase on the surface tension of BSA–Tween 20 mixed films as a function of protein/lipid ratio and temperature. Surface tension measurements were

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performed to determine the critical micelle concentration (CMC) of Tween 20 (polyoxyethylene sorbitan monolaurate) in water and aqueous solutions of ethanol and sucrose and to explore the existence of BSA–Tween 20 interactions. The influence of solutes in subphase on interfacial characteristics, such as surface dilational rheology (14) and drainage and diffusion in thin films (15) of BSA–Tween mixtures, has been studied recently. These results are of practical interest for food emulsions and foams generated by proteins in the presence of a water-soluble lipid, either added intentionally or present as an impurity. Moreover, an understanding of the micellization as a function of temperature is needed for a number of important industrial applications.

MATERIALS AND METHODS

Materials. The water used in this study was purified by means of a Millipore (Milford, MA) filtration device (Mille-Q). BSA (more than 96% pure, Fluka, Buchs, Germany), Tween 20 (polyoxyethylene sorbitan monolaurate, Fluka, Product No. 326896/1693), analytical grade ethanol (Merck, Darmstadt, Germany; >99.8%), sucrose (Fluka, >99.5%), potassium dihydrogen phosphate (99.5% pure, Merck), and dipotassium hydrogen phosphate (99% pure, Merck) were used as supplied.

Surface tension. Measurements were performed with a Krüss digital tensiometer K 10, based on the Wilhelmy method, with a roughened platinum plate. The method was detailed in Part 1 (9). All aqueous subphases were prepared in 50 mM phosphate buffer and adjusted to pH 7.0 (0.05 wt% sodium azide was added as antimicrobial agent). The experiments were carried out at temperatures ranging from 5 to 30°C. The temperature of the system was maintained constant within 0.5°C by a circulating Heto thermostat (Bikerod, Denmark). The Tween 20 solutions were placed in the dish and then in an enclosing box and were allowed to stand for 24 h to reach the desired adsorption equilibrium. Several stock solutions of the lipid with different concentrations were prepared and measured. To obtain more data points, some of the Tween 20 stock solutions were diluted with the same subphase after the first measurement. The CMC of Tween 20 in water and ethanol (0.5, 1.0, and 2.5 M), and sucrose (0.5 M) aqueous solutions was determined by surface tension measurements. Other experiments were done to study BSA–Tween 20 interactions at the air–water interface. BSA, dissolved in standard phosphate buffer solution (1% w/w, pH 7, ionic strength 0.05), was injected in the aqueous solutions under a lipid film previously adsorbed to the air–water interface to obtain a final BSA concentration of 0.1% w/w, and was allowed to stand for 48 h to reach the desired steady-state value in surface tension. Measurements were performed a minimum of five times. Surface tension measurements were reproducible within ± 0.5 mN/m.

RESULTS AND DISCUSSION

Interfacial characteristics of Tween 20 in aqueous solutions of ethanol and sucrose. Figures 1 to 5 show the results of the

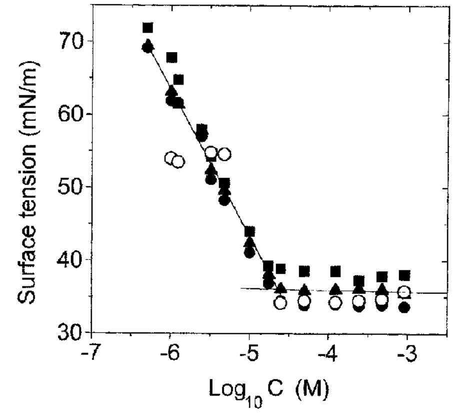

FIG. 1. Surface tension vs. logarithm of the molar concentration of Tween 20 (mol/L) in water in the presence (○) and absence (■, ▲, ●) of bovine serum albumin (BSA) at 0.1 wt%. Temperature: (■) 5, (○, ▲) 20, and (●) 30°C. The line shows the polynomial-fitted function.

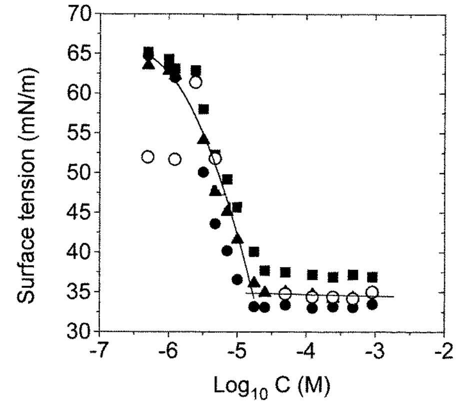

FIG. 2. Surface tension vs. logarithm of the molar concentration of Tween 20 (mol/L) in aqueous ethanol solution at 0.5 M, in the presence (○) and absence (■, ▲, ●) of BSA at 0.1 wt%. Temperature: (■) 5, (○, ▲) 20, and (●) 30°C. The line shows the polynomial-fitted function. For abbreviation see Figure 1.

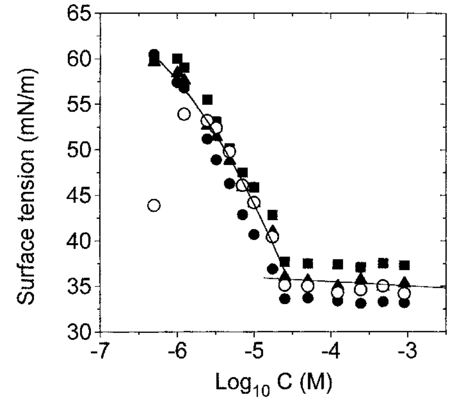

FIG. 3. Surface tension vs. logarithm of the molar concentration of Tween 20 (mol/L) in aqueous ethanol solution at 1.0 M, in the presence (○) and absence (■, ▲, ●) of BSA at 0.1 wt%. Temperature: (■) 5, (○, ▲) 20, and (●) 30°C. The line shows the polynomial-fitted function. For abbreviation see Figure 1.

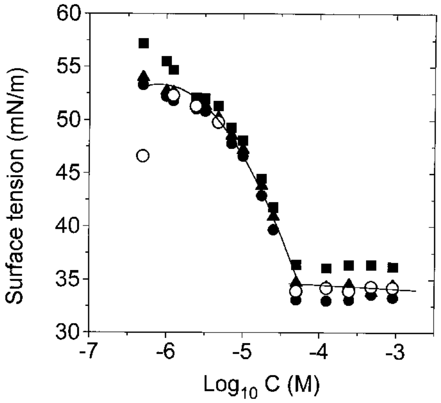

FIG. 4. Surface tension vs. logarithm of the molar concentration of Tween 20 (mol/L) in aqueous ethanol solution at 2.5 M, in the presence (○) and absence (■, ▲, ●) of BSA at 0.1 wt%. Temperature: (■) 5, (○,▲) 20, and (●) 30°C. The line shows the polynomial-fitted function. For abbreivation see Figure 1.

surface tension measurements for Tween 20 on water and aqueous solutions of ethanol and sucrose. Figure 1 shows the surface tension (σ) vs. $\log_{10}$ (concentration) for Tween 20 on water. Figures 2 to 4 show the surface tension (σ) vs. $\log_{10}$ (concentration) for Tween 20 in aqueous solutions of ethanol at 0.5, 1.0, and 2.5 M, respectively. The surface tension (σ) vs. $\log_{10}$ (concentration) for Tween 20 on sucrose aqueous solution at 0.5 M is shown in Figure 5. The curves of surface tension (σ) vs. $\log_{10}$ (concentration) for all systems studied are classical. Addition of low Tween 20 concentrations caused only a small reduction in surface tension, especially in aqueous solutions of ethanol and sucrose (Figs. 2 to 5). With increasing Tween 20 concentration, a sudden decrease in surface tension with the logarithm of the concentration was observed. When an excess of Tween 20 was added, the surface tension remained almost constant, as expected for a solution of a micellar surfactant. A break in the slope of surface tension vs. concentration usually indicates the formation of micelles. From these experimental data, the CMC and the surface tension at CMC, σ_{CMC}, can be deduced as a function of aqueous phase composition (Table 1). In Table 1, the data of CMC and σ_{CMC} for Tween 20 in NaCl aqueous solutions has been included for comparison (16). The CMC was obtained analytically by the cut point of the lines at low and high Tween 20 concentrations. Above the CMC, a straight line fits the data of surface tension vs. $\log_{10}$ (concentration) with a slope that is practically zero. This is an indication of the purity of the reagents used; the Tween 20 in sucrose aqueous solution at the highest temperature is an exception (Fig. 5). At lower Tween 20 concentrations than CMC, a second-order polynomial equation fits the data better. Only the fitted curves at 20°C are shown as lines in the figures to add clarity. The CMC value for Tween 20 in water at 20°C, determined here (Table 1), is slightly lower than that previously reported by Coke *et al.* (17) on a water solution with lower ionic strength ($I = 0.01$ M, $\text{CMC} = 3.5 \cdot 10^{-5}$ M), but it is difficult to compare our data directly with those of the literature because different lots of Tween 20 were used.

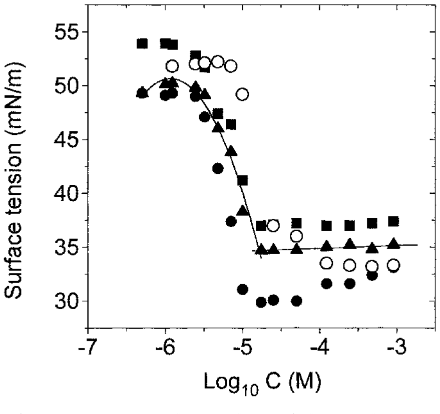

FIG. 5. Surface tension vs. logarithm of the molar concentration of Tween 20 (mol/L) in aqueous sucrose solution at 0.5 M, in the presence (○) and absence (■, ▲, ●) of BSA at 0.1 wt%. Temperature: (■) 5, (○, ▲) 20, and (●) 30°C. The line shows the polynomial-fitted function. For abbreviation see Figure 1.

From these experimental data we concluded that Tween 20 is able to form micelles in water and in aqueous solutions of ethanol and sucrose. The σ_{CMC} value decreases as the temperature is increased. The concentration that corresponds to the cut point was found to decrease with increasing temperature. That is, the surface activity of Tween 20 aqueous solutions decreases as temperature increases.

The interfacial characteristics of Tween 20 depend on the aqueous phase composition (Table 1). In ethanol aqueous solutions, the value of CMC increased as the ethanol concentration was increased, but the σ_{CMC} values were practically independent of the ethanol content in the aqueous phase. In contrast to this general behavior, the σ_{CMC} value decreased at the lower temperature and at the high concentration of ethanol (2.5 M). The effect of temperature on Tween 20 micelle formation was more complex—in aqueous ethanol solutions at 0.5 M, the CMC decreased as the temperature was increased, but it was practically independent of temperature at higher ethanol concentrations. So, the presence of ethanol in the aqueous phase breaks up micelle structure and results in a decrease in micelle size (18). The effect of alcohols on critical micelle concentration is well documented in the literature (18). Ethanol is a border case between short-chain and long-chain alcohols (with more than four carbons in the alcohol molecule), showing an increase of the CMC with ethanol concentration or presenting a minimum, depending on the surfactant. Because ethanol–water mixtures are better solvents for most surfactants than pure water, micelle formation occurs at higher surfactant concentrations, which is supported by data in Table 1.

In contrast, in sucrose aqueous solutions, the CMC decreased, but the interfacial activity of Tween 20 was similar to that in water—especially at 5 and 20°C. The addition of sucrose increases the surface tension of water, which indicates its strong cohesive force on water molecules, in addi-

TABLE 1
Critical Micelle Concentration (CMC, M) and Surface Tension at the CMC (σ_{CMC}, mN/m) for Tween 20 on Water and Aqueous Solutions of Ethanol, Sucrose, and Sodium Chloride (NaCl), as a Function of Temperature

Temperature	5°C		20°C		30°C	
Subphase	CMC ($M \times 10^5$)	σ_{CMC} (mN/m)	CMC ($M \times 10^5$)	σ_{CMC} (mN/m)	CMC ($M \times 10^5$)	σ_{CMC} (mN/m)
Water	1.95	37.7	1.69	35.0	1.08	33.0
Ethanol, 0.5 M	1.95	37.7	1.69	35.0	1.08	33.1
Ethanol, 1 M	2.47	37.7	2.59	35.9	2.41	33.6
Ethanol, 2.5 M	5.19	36.3	5.10	34.7	5.10	32.9
Sucrose, 0.5 M	1.65	37.0	1.32	34.7	1.10	30.2
NaCl, 0.1 M	2.93	39.6	4.32	36.3	2.88	35.6
NaCl, 0.5 M	1.01	38.8	0.95	36.4	0.90	33.9

tion to the induction of negative surface excess concentration or depletion of sucrose from the air–water interface. It is likely that these phenomena result in enhanced interactions between the Tween 20 molecules and also in the observed lower CMC values of Tween 20 on aqueous sucrose solution (Table 1). Another possible explanation for CMC to decrease in the presence of sucrose is the solubilization of sucrose in Tween 20 micelles in solution. In fact, solubilization is known to decrease CMC and increase the relative size of micelles in surfactant solutions.

The addition of NaCl increased the value of σ_{CMC} and lowered the CMC of Tween 20 at a salt concentration of 0.5 M, but it increased the CMC at a salt concentration of 0.1 M, as discussed previously (16). As with sucrose, addition of NaCl increased the surface tension of water. Moreover, small ions of high valency are highly hydrated, increase the organization of water molecules by compacting water molecules around themselves, and have negative or low standard molar entropies of hydration (19); and for these reasons they are called structure-making ions. Finally, NaCl in the aqueous phase could interact with the hydration sphere around the polar head group, and thus the hydrophobic character of Tween 20 increases (20). All of these phenomena could facilitate the micellization of Tween 20, which agrees with the lower CMC value of Tween 20, especially at the higher NaCl concentration (Table 1). The fact that inorganic electrolytes, such as NaCl, reduce the coalescence of gas bubbles above a critical concentration has generated controversy and resurgence in research on electrolyte effects on bubble coalescence. Surface tension, viscosity, and electrically repulsive forces were ruled out as possible explanations, with the preferred reasons being a reduction in the hydrophobic attraction between bubbles, the role of dissolved gases in the hydrophobic interactions and the Gibbs-Marangoni effect and surface elasticity due to the presence of electrolytes (19).

Interfacial excess concentration of Tween 20, Γ, can be estimated by applying the Gibbs thermodynamic approach (2,21) to the interfacial tension isotherm:

$$\Gamma = -\frac{1}{zRT} \bullet \frac{d\sigma}{d\mathrm{Ln}C} \qquad [1]$$

where the activity has been equated by the Tween 20 concentration (C) (assuming ideal behavior for all Tween 20 aqueous solutions investigated here), T is the absolute temperature (K), R is the gas constant, LnC is the natural logarithm of Tween 20 concentration, and $z = 1$ for nonionic surfactant (Tween 20). The term $d\sigma/d\mathrm{Ln}C$ was obtained by numerical differentiation of the interfacial tension isotherm by following Equation 1. The maximum surface excess (Γ_{max}) was calculated by Equation 1, where the term $(d\sigma/d\mathrm{Ln}C)_{CMC}$ was obtained at the CMC. The molecular area of Tween 20 at the maximum interfacial concentration, A_{CMC}, can be evaluated by:

$$A_{CMC} = \frac{1}{\Gamma_{max} \bullet N} \qquad [2]$$

where N is Avogadro's number. Table 2 shows the effect of aqueous phase composition and temperature on the maximum surface excess (Γ_{max}) and the area occupied by Tween 20 at CMC (A_{CMC}).

For two surface-active components—as is the situation for Tween 20 in aqueous ethanol solutions—the Gibbs equation for a multicomponent system can be used (22,23).

$$-\frac{d\sigma}{d\mathrm{Ln}C} = RT\sum_i \Gamma_i \qquad [3]$$

where Γ_1 and Γ_2 refer to the surface excess of Tween 20 and ethanol, respectively. A combination of the Langmuir isotherm and the Gibbs equation leads to a multicomponent form of the Szyskowski equation for the surface tension of mixed surfactants (22):

$$\Pi = \sigma_o - \sigma = RT\Gamma_{max} \bullet \mathrm{Ln}(1 + K \bullet C) \qquad [4]$$

where σ and σ_o are the surface tension of the system and the aqueous subphase surface tension, respectively, $K = K_1 \cdot \alpha_1 + K_2 \cdot \alpha_2$ for ideal mixing at the surface, K_1 and K_2 are the adsorption coefficients, and α_1 and α_2 are the macroscopic mole fractions for Tween 20 and ethanol, respectively. This equation is valid when there is no area change upon mixing, and it

TABLE 2
Interfacial Characteristics of Tween 20 Adsorbed on the Air-Aqueous Phase Interface, as a Function of Temperature[a]

Subphase	Temperature (°C)	Γ_{max} ($mol \cdot m^{-2}$)$\cdot 10^6$	A_{max} ($nm^2 \cdot molecule^{-1}$)	K ($m^3 \cdot mol^{-1}$)
Water	5	4.19	0.396	—
Water	20	3.56	0.466	—
Water	30	3.57	0.465	—
Ethanol, 0.5 M	5	3.15	0.527	2482
Ethanol, 0.5 M	20	3.89	0.427	1228
Ethanol, 0.5 M	30	4.58	0.362	1129
Ethanol, 1 M	5	4.01	0.414	380
Ethanol, 1 M	20	3.55	0.468	413
Ethanol, 1 M	30	3.30	0.503	546
Ethanol, 2.5 M	5	3.33	0.498	68.3
Ethanol, 2.5 M	20	3.61	0.460	68.3
Ethanol, 2.5 M	30	3.80	0.437	57.6
Sucrose, 0.5 M	5	5.05	0.329	—
Sucrose, 0.5 M	20	4.56	0.364	—
Sucrose, 0.5 M	30	4.98	0.333	—
NaCl, 0.1 M	5	5.96	0.279	—
NaCl, 0.1 M	20	5.85	0.284	—
NaCl, 0.1 M	30	2.71	0.613	—
NaCl, 0.5 M	5	9.01	0.184	—
NaCl, 0.5 M	20	8.70	0.191	—
NaCl, 0.5 M	30	8.78	0.189	—

[a] Γ, Gibbs thermodynamic approach; A, maximum interfacial area; K, adsorption coefficient.

is consistent with the ideal adsorbed solution adsorption. For molecules that differ greatly in size, the ideal mixing conditions may be not fulfilled (24,25). LeVan and Vermeulen (26) derived a set of thermodynamically consistent equations of which Equation 4 is a particular case. The adsorption coefficient (K) of the mixture, by following Equation 4, was calculated from the interfacial tension isotherms (Figs. 2–4) and is included in Table 2.

The maximum surface excess is higher for Tween 20 in aqueous solutions of sucrose and NaCl at every solute concentration in the bulk phase (Table 2)—the behavior of Tween 20 on aqueous solutions of 0.1 M NaCl at 30°C is an exception. As was previously discussed, molecular environmental factors that change the hydrophobic character of a lipid molecule affect the CMC. So, if the addition of sucrose and NaCl at higher concentrations increases the hydrophobic character of Tween 20, the high surface excess concentration of Tween 20 in these aqueous solutions is consistent with this fact (20).

The maximum Tween 20 surface excess in the presence of ethanol apparently does not depend on the ethanol concentration in the aqueous phase. We emphasized that the accurate determination of this parameter depends on the precision of the interfacial tension isotherm and on the fit of the experimental curves. The use of the empirical polynomial approximation creates the best fit for the interfacial tension isotherm (27–30). On the other hand, the constancy in the maximum Tween 20 surface excess in aqueous solutions of ethanol (Table 2) could be due to the competitive displacement of ethanol for Tween 20 from the interface. Accepting the cautions above, when an excess of ethanol exists in the aqueous phase and in the interface, the adsorption of each molecule of Tween 20, with a high affinity for the interface, must displace ethanol molecules previously adsorbed. This phenomenon agrees with the data of the adsorption coefficient included in Table 2: the value of the adsorption coefficient increases at the lower ethanol concentrations, which is an indication that the displacement of ethanol by Tween 20 from the interface increases at reduced ethanol concentrations in the bulk phase. Frances *et al.* (24) concluded, in agreement with data in Table 2, that larger molecules (Tween 20 in our work) may adsorb more than their bulk mole fraction if they have a larger adsorption coefficient (K_1) at low concentration. As the total concentration increases, however, and for steric and entropic reasons, the larger molecules can be displaced by the smaller molecules, which can eventually dominate the adsorption, provided that their size advantage is not overwhelmed by a disadvantage in the adsorption coefficient. Moreover, Lucassen-Reynders (25) suggests that any emulsifier can be displaced from the interface by another emulsifier added in sufficiently high concentration, but small emulsifier molecules are shown to be far more efficient at displacing large ones than vice versa.

The partial displacement of ethanol for Tween 20 at the interface could also be deduced from the interfacial tension isotherm for pure ethanol (Fig. 6): the interface is practically saturated by the ethanol at ethanol concentrations in the bulk phase of about 5 M. Moreover, at the maximum ethanol concentration studied here (2.5 M), the surface excess (Γ) is higher than the maximum surface excess of the Tween 20 adsorbed from aqueous ethanol solutions (Table 2). Such observations provide evidence for the possibility that, in aqueous solutions of ethanol, this solute as well as Tween 20 could be

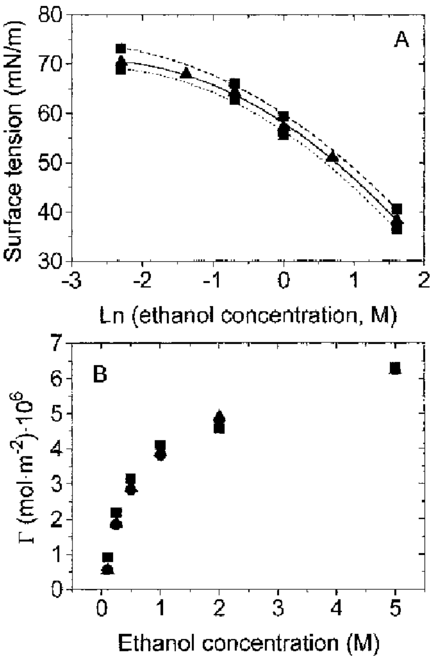

FIG. 6. Surface tension vs. logarithm of the molar concentration of ethanol (A) and maximum surface excess of ethanol (B), as a function of temperature: (■) 5, (▲) 20, and (●) 30°C. The lines show the polynomial-fitted functions.

adsorbed at the interface, and that the component that dominates the maximum superficial excess depends on the ethanol concentration in the aqueous phase.

Interfacial characteristics of BSA–Tween 20 mixed systems. When Tween 20 and BSA are both present in water or aqueous solutions of ethanol and sucrose, both emulsifiers will form adsorbed films at the air-water interface. The BSA–Tween 20 interactions at the interface can be deduced from the surface tension–Tween 20 concentration dependence (Figs. 1–5). The form of these curves is typical of many systems that are made up of protein + anionic surfactant (8,31) or protein + nonionic surfactant (6). In our experiments, the amount of BSA injected in the subphase under a film of previously adsorbed Tween 20 was maintained constant at 0.1 wt%. Therefore, the variation of the BSA/Tween 20 ratio is due to the variation of the Tween 20 concentration in the aqueous phase.

We deduced that the BSA–Tween 20 interactions depend on the BSA/Tween 20 ratio and on the aqueous phase composition. At the lower Tween 20 concentration—well below that of the CMC—the interfacial activity of the BSA–Tween 20 mixed systems was higher than that of the pure lipid. Figures 1 to 5 show that the σ values for the BSA–Tween 20 mixed systems are lower than for Tween 20 alone, no matter what the aqueous phase composition is. However, the surface tension was higher than that of the BSA alone on the same aqueous solution (Table 3); BSA–Tween 20 mixed films on aqueous ethanol solution at 1 M are an exception. This is an indication that in this region the protein predominates at the interface, but with a different conformation than that of pure BSA. Moreover, it is likely that the formation of a BSA–Tween 20 complex could take place in this region of reduced Tween 20 concentration. It is known that proteins and surfactants can interact in solution to form surfactant-protein complexes, which may have different properties than those of the pure protein, as was reviewed by Dickinson and Woskett (8) and by Bos *et al.* (6). Nonionic surfactants are known to interact poorly with soluble proteins unless specific hydrophobic binding sites exist where cooperative binding of the protein, followed by conformational changes, could take place. As a consequence of these interactions, the total amount of protein adsorbed could be reduced by steric effects. It is possible therefore that a BSA–Tween complex formed in the aqueous phase can be adsorbed in a different conformation than the pure protein, resulting in a positive or negative effect on the adsorbed amount (6). This agrees with the data in Table 3.

From the surface tension data of BSA–Tween 20 mixed systems at Tween 20 concentrations below the CMC (Table 3), it appears that the interfacial activity depends on the aqueous phase composition. The interfacial activity of BSA–Tween 20 mixed systems in this region was higher for aqueous solutions of ethanol and decreased for aqueous solutions of sucrose, for water, and for aqueous solutions of NaCl, in this order. This phenomenon will be discussed later.

At Tween 20 concentrations above the CMC, the interfacial activity of the BSA–Tween 20 mixed systems was practically the same as that for pure Tween 20. Under these conditions, it is expected that the surface is covered with Tween 20, and the aqueous bulk phase could contain Tween 20 monomers and micelles, BSA, and probably BSA–Tween 20 complexes, but without any effect on the interfacial activity of the system. In this region, the surface activity of the mixed films did not depend on the aqueous phase composition. This is an indication that the structure of the adsorbed Tween 20 film is essentially the same for all subphases studied here. This behavior is different from that of spread insoluble lipids described in the preceding paper (9). In those situations, the monolayer structure depends greatly on the presence of ethanol (32), sucrose (33), or electrolytes (34) in the aqueous subphase.

TABLE 3
Surface Tension of Bovine Serum Albumin (BSA, 0.1%, w/w) and Mixed BSA (0.1%, w/w)–Tween 20 Films Adsorbed on the Air–Aqueous Phase Interface, at Tween 20 Concentrations (*C*, M) Below and Above the CMC (M). Temperature: 20°C

Subphase	BSA–Tween 20 σ (mN/m) *C* << CMC	BSA–Tween 20 σ (mN/m) *C* > CMC	σ (nN/m) BSA (0.1%, w/w)
Water	53.5–54.8	34.5–35.8	51.1
Ethanol, 0.5 M	50.0–51.4	34.2–35.0	48.6
Ethanol, 1 M	43.9	34.2–35.1	45.3
Ethanol, 2.5 M	46.6	33.9–34.2	44.8
Sucrose, 0.5 M	51.8–52.2	33.3–33.5	48.9
NaCl, 0.1 M	54.8–55.1	34.8–35.5	49.4
NaCl, 0.5 M	54.0–54.3	34.6–35.1	45.0

At Tween 20 concentrations closer to the CMC, the situation is more complex. The curve in the presence of BSA crossed that of pure Tween 20, which indicates the formation of a complex between the two emulsifiers because the crossover could be due to a reduction in the concentration of free Tween 20 in solution, which interacts with the BSA to form complexes (6). In this region, the surface tension falls more rapidly with increasing Tween 20 concentration than in the preceding region at a lower Tween 20 concentration. Dickinson and Woskett (8) suggested that the increasing occupation of surface sites by the adsorption of surfactant molecules between adsorbed tails of proteins and the existence of a protein–surfactant complex with a more or less hydrophobic character than the native protein could be the cause of the behavior observed. Because proteins contain hydrophobic and electrostatically charged groups, they can bind polar lipids by means of electrostatic or polar interactions—with Tween 20, as a polar lipid, the electrostatic interactions are rather weak—followed by hydrophobic interactions (6,35,36). The effects observed also suggest that proteins and lipids may interact with the interface through solubilization or replacement mechanisms, depending on the interactions of lipids at the interface and protein–lipid binding by means of molecular mechanisms, which are not fully understood (6).

For BSA–Tween 20 mixed systems on water (Fig. 1) and aqueous solutions of sucrose (Fig. 5), the surface tension of mixtures was higher than that of Tween 20 alone, although in the mixed system the concentration of emulsifiers was higher. Again, this is attributable to BSA–Tween 20 interactions, which displace the protein adsorbed from the interface or induce conformational changes in the protein molecule and lead to a decreased surface activity. The BSA–Tween 20 mixed systems behaved differently in aqueous solutions of ethanol. As shown in Figures 2 to 4, the surface activity of the mixed systems was the same as that of Tween 20 alone. This behavior was observed for Tween 20 concentrations in the bulk phase below the CMC by one or two orders of magnitude, especially as the ethanol concentration in the aqueous phase was increased. The phenomenon reported here could be associated with the effect of ethanol on the micellization of Tween 20, as was discussed in the preceding section. On the other hand, ethanol may be able to occupy hydrophobic binding sites of the protein molecule, and the hydrophilic head group of ethanol may confer solubility in water on the resulting complex.

As stated in the preceding paper (9), surface tension concentration is not sufficient to allow conclusions on the nature of a specific protein–lipid complex at the interface, especially if a reliable molecular interpretation is of interest. However, the surface tension is sensitive to protein displacement by lipids, depending on the protein–lipid system and on the nature of the aqueous solution. Further evidence for the behavior of mixed BSA–Tween 20 systems as a function of protein/lipid ratio and aqueous phase composition arises from recent results about surface dynamic properties—such as dynamic surface tension and surface rheological characteristics (14) and drainage and superficial diffusion for the same systems studied here (15). We found a sudden drop in surface dilational modulus after addition of Tween 20 to BSA aqueous solutions, which reached a minimum at a characteristic Tween 20 concentration of the same order of magnitude but lower than the CMC, and that ultimately increased to a plateau value as the Tween 20 concentration increased. For the same systems, the drainage behavior and superficial diffusion were also remarkably different from that of pure protein or pure lipid film, and again the transition occurred at Tween 20 concentrations close to the CMC.

The results of this investigation imply that solutes in the aqueous phase play a role in Tween 20 micellization and BSA–Tween 20 interactions. Generally, BSA and Tween 20 interact at the interface and in the bulk phase. At low Tween 20 concentrations, BSA reduces the surface tension to a greater extent than BSA–Tween 20 mixed systems. However, the opposite was observed at high Tween 20 concentrations, above the CMC, because the BSA molecules are displaced from the interface by the Tween 20. Over the intermediate region, close to the CMC, both BSA and Tween 20 coexist at the interface. In this regard, BSA-insoluble spread lipids, such as monostearin and monoolein (12,13) and BSA-soluble Tween 20 systems, behave in a similar way. The intermediate region is of practical interest for food emulsions and foam formulations because the stability of the system is greatly affected (17,37–39). The origin of this instability may rest in the incompatibility of the two stabilization mechanisms: the Gibbs-Marangoni mechanism, involving lateral diffusion of lipid molecules at the interface, and the viscoelastic mechanisms on immobilization of the protein molecules at the interface (7). It has been demonstrated that aqueous phase composition plays a role in the existence of BSA–Tween 20 interactions and, as consequence, on the interfacial characteristics of Tween 20 and mixed BSA-Tween 20 films. It is likely that this is due to the effect of solutes in the subphase on the competitive adsorption of BSA, Tween 20, and BSA–Tween 20 complex, and on the BSA structure at the interface and in the bulk phase.

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