

Rheokinetic Analysis of Bovine Serum Albumin and Tween 20 Mixed Films on Aqueous Solutions

M. Rosario Rodríguez Niño,[†] Peter J. Wilde,[‡] David C. Clark,^{†,‡} and Juan M. Rodríguez Patino^{*,†}

Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, c/Prof. García González s/núm, 41012 Sevilla, Spain, and Institute of Food Research, Food Biophysics Department, Norwich Laboratory, Norwich Research Park, Norwich NR4 7UA, United Kingdom

The surface dynamic properties (surface tension and surface dilational properties) of films formed by bovine serum albumin (BSA) and the surfactant Tween 20 were studied using periodic sinusoidal interfacial compression and expansion in a superficial rheometer (ring trough). All measurements were performed at a constant temperature (20 °C) and at constant BSA concentration in the bulk phase (0.1% w/w). The surface dynamic properties were dependent on the protein/surfactant ratio in the bulk phase, on the interfacial composition (BSA, Tween 20, and BSA–Tween 20 mixture), and on the aqueous phase composition (i.e., presence of ethanol and sucrose). The results show that at higher protein concentrations in mixed films the surface dilational modulus is higher, which agrees with greater protein–protein interactions. However, lower superficial dilational modulus was observed at higher surfactant concentrations, which agrees with weaker surfactant–surfactant interactions. The dynamic surface tension and surface dilational modulus depend on subphase composition. The surface dynamic properties were tested by a kinetic model to monitor the unfolding and rearrangements of adsorbed BSA molecules including the existence of BSA–Tween 20 interactions.

Keywords: *Superficial dilational rheology; adsorption; surface properties; bovine serum albumin; Tween 20*

INTRODUCTION

Food colloids (foams or emulsions) are thermodynamically unstable systems. Usually the two fluids present are immiscible, so energy has to be put in to achieve the dispersion of the system (Walstra, 1983, 1987). However, the lifetime of a dispersed system composed of two pure, immiscible fluids is very short. For this reason, an emulsifier has to be present to facilitate the efficient production of new interface between the two phases (Dickinson, 1989; Halling, 1981). Food colloids are essentially stabilized by low molecular weight emulsifiers, by macromolecules, or by a mixture of both of these emulsifiers (Charalambous and Doxastakis, 1989; Dickinson, 1992; Larsson and Friberg, 1990; Leadbetter, 1990). Because of their amphiphilic structure with both polar and nonpolar parts, emulsifiers tend to orient themselves at the oil–water or air–water interface, lowering the interfacial or superficial tension and conferring stability to the dispersed phase by forming a protective adsorbed layer around the droplet or bubble surface. Knowledge of the characteristics of emulsifier films (structure, stability, rheological characteristics, etc.) being either adsorbed or spread at fluid–fluid interfaces is particularly important, since these characteristics are directly linked to the overall

stability behavior and properties of the final product (Dickinson, 1989). Moreover, the presence of protein–surfactant interactions can have a pronounced impact on the interfacial behavior of the components (Bos et al., 1997).

The formation of the interfacial layer and its subsequent physicochemical properties are strongly affected not only by the interfacial composition (protein, surfactant or their mixtures) but also by the properties and composition of the bulk phase (Rodríguez Niño, 1998; Rodríguez Niño et al., 1996, 1997a,b; Rodríguez Patino et al., 1992, 1993, 1997). In this work we studied the surface dynamic properties—such as surface tension and surface dilational properties (surface dilational modulus, elastic and viscous components, and loss angle tangent)—of adsorbed films at the air–water interface, formed by a protein [bovine serum albumin (BSA)], a soluble surfactant (Tween 20), and its mixtures. The protein/surfactant ratio and the aqueous phase composition (1 M ethanol and 0.5 M sucrose) were the variables studied.

MATERIALS AND METHODS

Materials. BSA (Fluka Chemie AG, Boch, Germany, >96% pure), Tween 20 (polyoxyethylene sorbitan monolaurate, Fluka, high purity), analytical grade ethanol (Merck, Darmstadt, Germany, >99.8%), sucrose (Fluka, >99.5%), potassium dihydrogenphosphate (Merck, 99.5%), and dipotassium hydrogenphosphate (Merck, 99%), were used without further purification. All samples were prepared using double-distilled, surface chemically pure water.

Method. The surface rheological parameters—such as surface dilational modulus, elastic and viscous components, and loss angle tangent—and the surface tension were mea-

* Author to whom the correspondence should be addressed (fax + 34 5 4557134; telephone +34 5 4557183; e-mail jmrodri@cica.es).

[†] Universidad de Sevilla.

[‡] Norwich Laboratory.

[§] Present address: DMV International, NCB-laan 80, P.O. Box 13, 5460 BA Veghel, The Netherlands.

sured according to the method of Kokelaar et al. (1991) as a function of time and radial frequency, as described in detail elsewhere (Rodríguez Niño et al., 1996). The method involves a modified Langmuir trough using a cylindrical barrier of internal area, A , of 70.9 cm² to induce a periodic sinusoidal interfacial expansion and compression of the surface. The percentage area change was 6%, which was determined to be in the linear region. The surface dilational modulus, E , derived from the small change in surface tension ($d\sigma$), measured by using a homemade glass Wilhelmy plate, which is in permanent contact with the liquid surface, resulting from a small change in surface area, dA , may be described by the equation (Lucassen and van den Temple, 1972)

$$E = d\sigma/(dA/A) = -d\pi/d \ln A \quad (1)$$

where $\pi = \sigma_0 - \sigma$ is the surface pressure and σ_0 is the subphase surface tension.

The surface dilational modulus is a complex quantity and is composed of real and imaginary parts, $E = |E|(\cos \varphi + i \sin \varphi)$, where $|E|$ represents the amplitude ratio between surface stress and strain and φ is the loss angle of the modulus. The real part of the dilational modulus or storage component is the dilational elasticity, $E_d = |E| \cos \varphi$. The imaginary part of the dilational modulus or loss component, $E_v = |E| \sin \varphi$, is related to the product of surface dilational viscosity, η_d (mN·s·m⁻¹), and the radial frequency ω (rad·s⁻¹), $E_v = \eta_d \cdot \omega$. The loss angle tangent can be defined as $\tan \varphi = E_v/E_d$. If the film is elastic, the loss angle tangent is 0. φ is the loss angle of the modulus, and ω is the frequency of the oscillation.

All experiments were carried out at 20 °C. All of the aqueous subphases were prepared in 50 mM phosphate buffer and adjusted to pH 7.0. Aqueous solutions of 1 M ethanol and 0.5 M sucrose were studied as variables. The measurements were made as a function of Tween 20 concentration. To study BSA–Tween 20 interactions at the air–water interface, Tween 20 was added to a protein solution (0.1% w/w) in the concentration range of 0–30 μM. For experiments involving BSA or BSA–Tween 20 solutions, the emulsifier solutions were prepared at 20 °C by stirring for 30 min and then placed in the trough. Time began at the moment the BSA or BSA–Tween 20 solution (200 mL) was placed in the trough at 20 °C. Surface measurements are very sensitive to the presence of impurities, so extreme care was taken to ensure that all materials and equipment used in this study were clean as described elsewhere (Rodríguez Niño et al., 1998). The reproducibility of the results was better than 6%.

RESULTS AND DISCUSSION

Transient Surface Dynamic Properties. The dynamic surface tension and surface dilational properties for mixed BSA–Tween 20 films on water and aqueous solutions of 1 M ethanol and 0.5 M sucrose are shown in Figures 1–3, respectively. Different BSA–Tween 20 ratios behaved in a similar manner (data not shown). The time-dependent surface tension of BSA–Tween 20 (Figures 1–3) adsorbed films are typical. The surface tension decreased and the surface dilational properties—especially surface dilational modulus and surface dilational elasticity—increased with time and tended to a plateau. However, the rate of surface tension decrease or the rate of surface dilational modulus increase depended on the aqueous phase composition. As with the surface tension time dependence, the rate of increase of the surface dilational properties as deduced from the slope of E or σ with time (data not shown)—was highest in the presence of 0.5 M sucrose, then water, and, finally, 1.0 M ethanol.

If the surface transient properties are associated with BSA and/or BSA–Tween 20 adsorption at the air–water interface (Damodaran and Song, 1988; Graham and

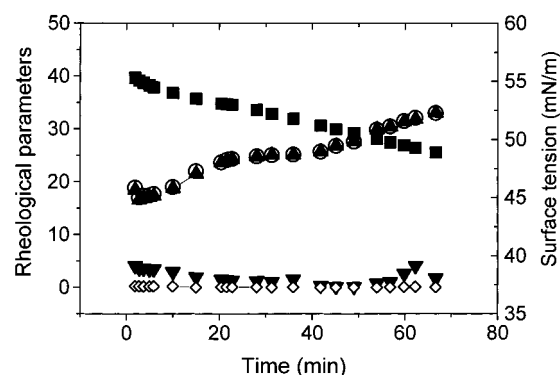


Figure 1. Time dependence of surface tension (σ , ■, mN/m), surface dilational modulus (E , ○, mN/m), elastic component (E_d , ▲, mN/m), viscous component ($\eta_d \cdot \omega$, ▼, mN/m), and loss angle tangent ($\tan \theta$, ◇) for BSA–Tween 20 adsorbed films on water at an angular frequency of 0.81 rad·s⁻¹. $T = 20$ °C. BSA concentration = 0.1% w/w. Tween 20 concentration = 7.5 μM.

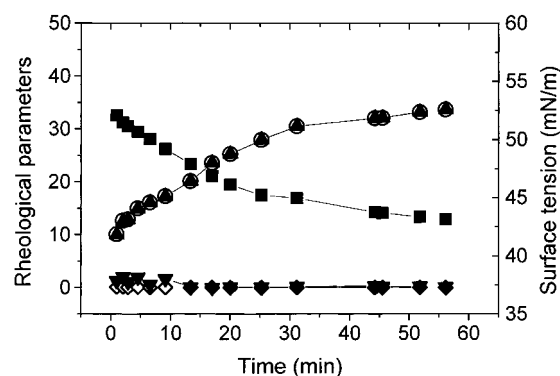


Figure 2. Time dependence of surface tension (σ , ■, mN/m), surface dilational modulus (E , ○, mN/m), elastic component (E_d , ▲, mN/m), viscous component ($\eta_d \cdot \omega$, ▼, mN/m), and loss angle tangent ($\tan \theta$, ◇) for BSA–Tween 20 adsorbed films on a 1.0 M ethanol aqueous solution at an angular frequency of 0.81 rad·s⁻¹. $T = 20$ °C. BSA concentration = 0.1% w/w. Tween 20 concentration = 7.5 μM.

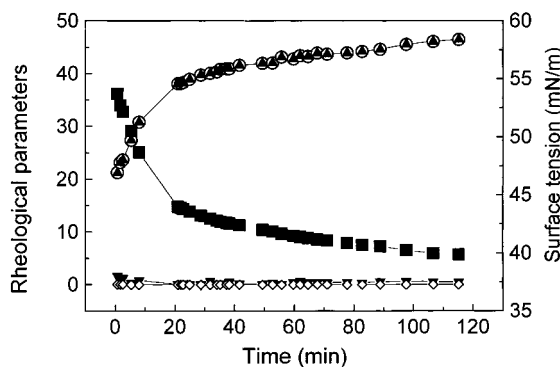


Figure 3. Time dependence of surface tension (σ , ■, mN/m), surface dilational modulus (E , ○, mN/m), elastic component (E_d , ▲, mN/m), viscous component ($\eta_d \cdot \omega$, ▼, mN/m), and loss angle tangent ($\tan \theta$, ◇) for BSA–Tween 20 adsorbed films on a 0.5 M sucrose aqueous solution at an angular frequency of 0.81 rad·s⁻¹. $T = 20$ °C. BSA concentration = 0.1% w/w. Tween 20 concentration = 7.5 μM.

Phillips, 1979), the rheokinetic data shown in Figures 1–3 can be related with the competitive or cooperative adsorption of BSA and Tween 20 at the interface, as a function of subphase composition. The fact that the time dependence of the surface dilational modulus and surface tension follows the same trend could indicate that E depends on the surface coverage, which is

Table 1. Characteristic Parameters for Adsorption of BSA–Tween 20 Mixtures on Water and Aqueous Solutions of 1 M Ethanol and 0.5 M Sucrose at 20 °C

subphase	Tween 20 concn (μM)	$k_1 \times 10^3$ min ⁻¹ (LR)	θ_1^* (min)	$k_2 \times 10^3$ min ⁻¹ (LR)	θ_2^* (min)	$k_3 \times 10^3$ min ⁻¹ (LR)	θ_{max} (min)
water ^a	0	56.7 (0.989)	50	39.5			62
water	5	110.2 (0.981)	9.5	43.7 (0.997)	49	133 (0.904)	61
water	7.5	17.1 (0.991)	30.7	44 (0.990)	57.6	255 (0.985)	66.6
water	10	22.4 (0.996)	19.4	67.4 (0.999)	45.2	143 (0.995)	57.3
water	15	35.2 (0.998)	29.7	70.2 (0.997)	50.1	176 (0.994)	63
water	20	81.4 (0.997)	17	50.0 (0.997)	43.3	138 (0.973)	61.3
water	30	187 (0.995)	5.3	56.0 (0.999)	54	155 (0.990)	60.7
1 M ethanol ^a	0	29.3 (0.973)	63	23.8			70
1 M ethanol	1	160 (0.986)	7.7	41.3 (0.993)	34.4	125 (0.988)	62.1
1 M ethanol	7.5	60.0 (0.996)	36.5	95.8 (0.996)	49.5	263 (0.986)	61.1
1 M ethanol	10	92.7 (0.996)	23.5	19.9 (0.960)	40.5	170 (0.992)	59.3
1 M ethanol	15	128 (0.997)	23.8	57.7 (0.977)	40.3	300 (0.958)	60.3
1 M ethanol	20	178 (0.997)	15.7	53.8 (0.983)	39.8	507 (0.972)	60.5
1 M ethanol	30	133 (0.993)	4.6	81.3 (0.998)	36	217 (0.963)	60
0.5 M sucrose ^a	0	33.6 (0.973)	80	-10.2			92
0.5 M sucrose	1	152 (0.956)	5.5	40.1 (0.989)	60.2	628 (0.891)	66
0.5 M sucrose	7.5	32.3 (0.997)	47	125 (0.990)	57.5	597 (0.948)	60.4
0.5 M sucrose	10	49.9 (0.995)	44.6	79.6 (0.934)	65.6	433 (0.983)	74.2
0.5 M sucrose	15	59.4 (0.999)	21.6	25.4 (0.998)	106.1	448 (0.979)	115.1
0.5 M sucrose	20	129 (0.995)	6.3	54.6 (0.995)	48.3	202 (0.982)	60.4
0.5 M sucrose	30	166 (0.979)	4.1	54.6 (0.985)	52.1	132 (0.964)	64.4

^a From Rodríguez Patino and Rodríguez Niño (1995a).

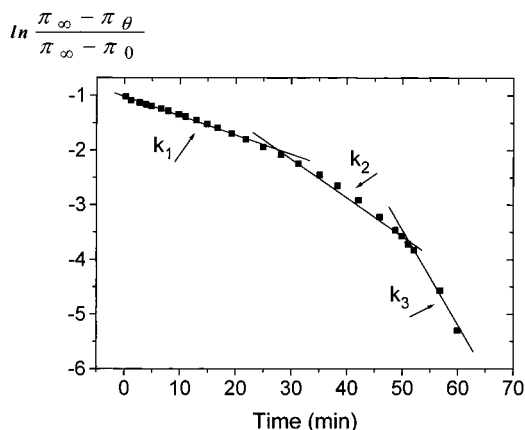


Figure 4. Rate of BSA–Tween 20 adsorption at the air–water interface. BSA concentration = 0.1% w/w. Tween 20 concentration = 15 μM . $T = 20\text{ }^\circ\text{C}$.

expected to increase with time (MacRitchie, 1989; Joos et al., 1992; Damodaran and Song, 1988). Due to this similarity, we propose an analogy to define a first-order kinetic equation for BSA–Tween 20 adsorption, eq 2,

$$\ln(\pi_\infty - \pi_\theta)/(\pi_\infty - \pi_0) = -k\theta \quad (2)$$

similar to that used by Tornberg (1978) and Graham and Phillips (1979), to monitor unfolding at the surface and configurational rearrangements of adsorbed protein molecules. As with other methods (Rodríguez Patino and Rodríguez Niño, 1995), the diffusion step is too fast to be detected with the experimental device use in this work. In eq 2, π_∞ , π_0 , and π_θ are the surface pressures at steady-state conditions, at time $\theta = 0$, and at any time, θ , respectively, and k is the first-order rate constant.

An example of the application of eq 2 to monitor the kinetics of BSA–Tween 20 adsorption at the air–water interface—in the period after that affected by diffusion—is shown in Figure 4. We find, for all the experiments of BSA–Tween 20 adsorption, three linear regions over the time interval studied. To summarize the effect of aqueous phase composition on the kinetics of BSA–

Tween 20 adsorption, the first-order rate constants derived from eq 2 are shown in Table 1. The fit of experimental data to the model was made at a time interval based on the best linear regression coefficient (LR), which is also included in Table 1. The characteristic time (θ_i^*) corresponding to a change in mechanism was obtained analytically as the cut-point of the linear fit corresponding to each step in the adsorption mechanism.

For pure protein the initial slope is taken to correspond to a first-order rate constant of unfolding (k_1), while the second slope is taken to correspond to a first-order rate constant of rearrangement (k_2), occurring among a more or less constant number of adsorbed molecules (Tornberg, 1978; Graham and Phillips, 1979; Suttiprasit et al., 1992). The problem in applying this equation to BSA–Tween 20 adsorbed films is that the BSA and Tween 20 could be adsorbed either as individual molecules or as a BSA–Tween 20 complex. Moreover, Tween 20 can form aggregates once a certain concentration, the critical micelle concentration (cmc), is reached. The study of soluble emulsifiers above the cmc is of practical importance because in many real food formulations the emulsifier concentration exceeds the cmc, and so monomer and micelle are likely to be present in the bulk phase competing with protein for the interface.

The analysis of the effect of aqueous phase composition on BSA–Tween 20 adsorbed films can be made on the basis of the molecular model drawn in Figure 5. So far, all theoretical models are based on surfactant solution with a distribution of surfactant molecules as monomers. The presence of micelles in solution can influence the adsorption kinetics remarkably and also the BSA–Tween 20 interactions. To account for the micelle effect, numerous papers on the determination of aggregation numbers and rate constants of micelle kinetics of many surfactants have been published (Chu et al., 1994; Dukhin et al., 1995). If we adopt first-order kinetics of micelle formation–disintegration according to Zana (1995) and Anthony and Zana (1996a,b), the first-order kinetic constants (k_i) obtained by fitting the experimental data according to eq 2 must be dependent

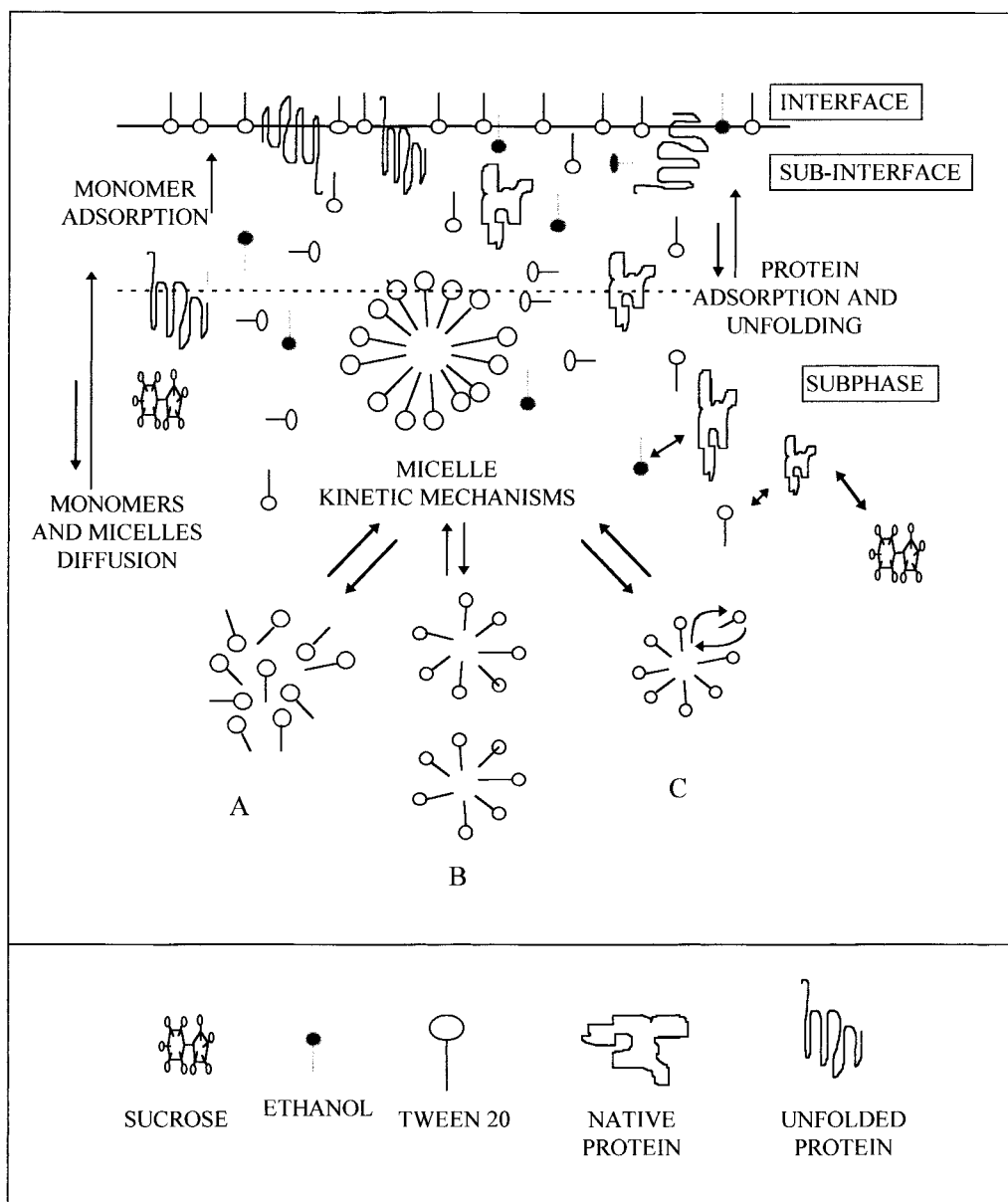


Figure 5. Schematic representation of the adsorption of BSA-Tween 20 mixed films from water and aqueous solutions of ethanol and sucrose in the presence or absence of micelles. Micelle kinetics mechanisms: (A) formation-dissolution; (B) rearrangement; (C) stepwise aggregation-disintegration.

on the BSA-Tween 20 adsorption process for monomers and micellar solutions of Tween 20, which agrees with the complex dependence between the variables studied (Table 1).

As a general rule, it can be seen (Table 1) that the individual steps for the rate of BSA-Tween 20 adsorption depend on both the Tween 20 concentration and the aqueous phase composition. The values of the first-order rate constant k_1 for BSA-Tween 20 mixed films decreased after Tween 20 addition, reached a minimum at Tween 20 concentration of $\sim 7.5 \mu\text{M}$, and then increased and tended to a plateau value at higher Tween 20 concentrations. The values of k_1 also depend on the aqueous phase composition. In fact, at Tween 20 concentration ranging between 7.5 and $20 \mu\text{M}$ the first-order rate constant is highest for the ethanol solution followed by the sucrose solution and lowest for water. This phenomenon is opposite that observed for adsorption of pure BSA films on the same aqueous solutions (Rodríguez Patino and Rodríguez Niño, 1995a). There-

fore, we can speculate that during the first step of the adsorption process, Tween 20 could be the dominant factor. The higher rate of surfactant adsorption as compared with that of protein adsorption is well documented in the literature (Damodaran and Song, 1988; Dukhin et al., 1995; Graham and Phillips, 1979).

The Tween 20 concentration dependence on the first-order rate constant for BSA-Tween 20 adsorption, k_2 (Table 1), is different from that of k_1 . In the presence of ethanol or sucrose, k_2 increased with Tween 20 concentration, described a maximum at a Tween 20 concentration of $\sim 7.5 \mu\text{M}$, and then decreased and tended to a plateau at higher Tween 20 concentrations. However, k_2 values for BSA-Tween 20 adsorption on water are not particularly dependent on the Tween 20 concentration.

Finally, the first-order rate constant k_3 for BSA-Tween 20 adsorption on the sucrose solution decreased as the Tween 20 concentration increased, a phenomenon opposite that observed in the presence of ethanol.

Moreover, the k_3 values for the adsorption of mixed films on water do not depend on the Tween 20 concentration. It must be emphasized that the first-order rate constant k_3 is 2–3 times higher than k_1 or k_2 independent of the aqueous phase composition.

Values of the first-order rate constants k_2 and k_3 do not agree with the control experiments involving pure BSA or pure Tween 20 films. Therefore, it can be concluded that interactions between the emulsifiers at the interface and possibly in the bulk phase may exist and affect the adsorption process of BSA–Tween 20 mixed films (see molecular model in Figure 5). These interactions are in turn influenced by the aqueous phase composition. The existence of these interactions (Figure 5) makes the analysis of the adsorption of mixed emulsifiers difficult. This is due to the fact that the different mechanisms schematized in Figure 5 could be consecutive or concurrent processes, which in turn depend on the BSA–Tween 20 ratio and the bulk phase composition. The existence of these interactions either at interface or in the bulk phase as well is documented in the literature (Clark et al., 1993; Coke et al., 1990; Cornell and Carroll, 1985; Erickson and Hegg, 1985; Bos et al., 1997). In general, surfactant and proteins may interact with interfaces by complex formation or displacement mechanisms, depending on surfactant surface interactions and surfactant–protein bonding. Moreover, proteins can interact with surfactants in solution as individual molecules or as micelles at higher surfactant concentrations above the cmc. The presence of solutes in the bulk phase is expected to affect the dynamics of micellar solutions as was reviewed recently by Zana (1995). Therefore, discussion on the adsorption kinetics of protein–surfactant mixtures at the air–aqueous phase interface can only be tentative at present. Clearly, more systematic experimental work needs to be carried out on mixed systems containing protein and surfactant to account for the kinetics of adsorption of these complicated systems.

The higher rate of BSA–Tween 20 adsorption in the presence of sucrose could be associated with the effect of sucrose either by direct interaction on protein structure in the bulk phase or by indirect interaction through modification of the solvent environment or by a combination of both effects (Boye et al., 1996a,b; Crowe et al., 1987; Ismond et al., 1988; Lee and Timasheff, 1981). If sucrose is present in the aqueous solution, the protein is preferentially hydrated with a native structure and lower protein–protein interactions. These phenomena allowed more protein to be involved in film formation, as was deduced from tensiometry (Rodríguez Patino and Rodríguez Niño, 1995a,b) and surface dilational properties (Rodríguez Niño et al., 1997b). The lower rate of BSA–Tween 20 adsorption in the presence of ethanol could be associated with the competitive adsorption of ethanol, BSA, and Tween 20. That is, BSA and Tween 20 can compete with ethanol molecules previously adsorbed due to their higher affinity for the interface, as a consequence of their greater hydrophobicity.

Surface Rheological Dilational Properties. From a rheological point of view, it must be emphasized that the values of the surface dilational modulus were similar to that of surface dilational elasticity, either for BSA–Tween 20 adsorbed films on water (Figure 1) or for aqueous solutions of 1 M ethanol (Figure 2) or 0.5 M sucrose (Figure 3). The values of surface dilational viscosity were low for BSA–Tween 20 mixed films. The

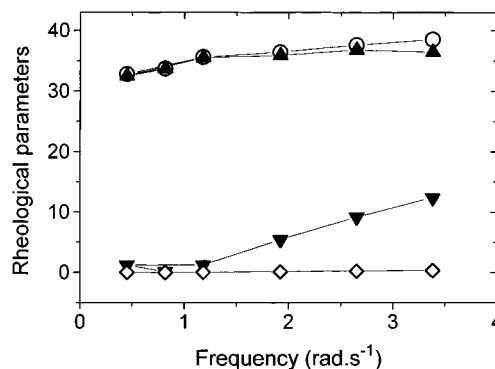


Figure 6. Angular frequency ($\text{rad}\cdot\text{s}^{-1}$) dependence of surface tension (σ , ■, mN/m), surface dilational modulus (E , ○, mN/m), elastic component (E_a , ▲, mN/m), viscous component ($\eta_a\omega$, ▼, mN/m), and loss angle tangent ($\tan \theta$, ◇) for BSA–Tween 20 adsorbed films on water. $T = 20^\circ\text{C}$. BSA concentration = 0.1% w/w. Tween 20 concentration = $7.5\ \mu\text{M}$.

loss angle tangent was practically 0. The frequency dependence of rheological parameters (Figure 6) characterizes a viscoelastic behavior of the surface over the range of frequencies studied, which is essentially elastic. Different BSA–Tween 20 ratios on different aqueous subphases behaved in a similar manner (data not shown).

The surface dilational properties also depended on the aqueous phase composition. On water and aqueous sucrose solutions, the surface dilational properties—especially surface dilational modulus and surface dilational elasticity—were lower for BSA–Tween 20 mixed films than for pure BSA films (Rodríguez Niño et al., 1997a). However, on ethanol aqueous solutions the surface dilational modulus for BSA–Tween 20 films was higher than that for pure BSA films (Rodríguez Niño et al., 1997a). This phenomenon is opposite that observed with pure BSA films adsorbed on water, in which case the presence of solutes (ethanol or sucrose) produced a significant decrease in the surface dilational modulus, which can be dramatic for BSA films on ethanol aqueous solutions (Rodríguez Niño et al., 1997a). Therefore, the positive synergistic effect of Tween 20 on surface rheological properties of BSA–Tween 20 mixed films must be emphasized for practical reasons, especially for mixed films adsorbed on ethanol aqueous solutions.

Effect of Tween 20 Concentration. The effect of Tween 20 concentration on surface tension and surface dilational properties of BSA–Tween 20 mixed films adsorbed on water and aqueous solutions of ethanol and sucrose are shown in Figures 7–9, respectively. The surface tension decreased markedly as the amount of Tween 20 in the bulk phase increased, but it tended to a plateau value as the concentration increased.

The Tween 20 concentration dependence on the surface dilational modulus was more complex. The effect depended on the type of solute in the subphase. On water (Figure 7) a sudden drop in surface dilational modulus existed after addition of Tween 20, reaching a minimum at $5\ \mu\text{M}$ Tween 20, and ultimately increased to a plateau value as the Tween 20 concentration increased above $20\ \mu\text{M}$. On an ethanol aqueous solution (Figure 8), the surface dilational modulus increased with Tween 20 concentration and tended to a plateau value at Tween 20 concentration of $\sim 10\ \mu\text{M}$. On a sucrose aqueous solution (Figure 9), the effect of Tween 20 was similar to that observed on water. That is, the surface

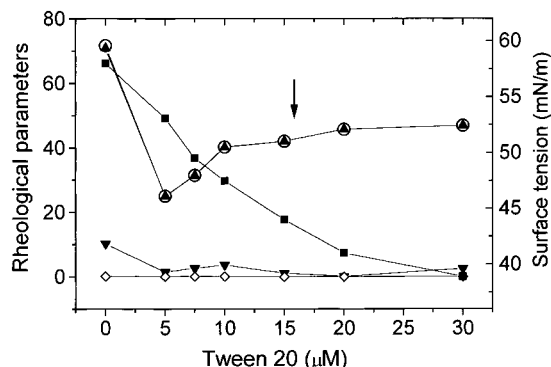


Figure 7. Surface tension (σ , ■, mN/m), surface dilational modulus (E , ○, mN/m), elastic component (E_d , ▲, mN/m), viscous component ($\eta_d \omega$, ▼, mN/m), and loss angle tangent ($\tan \theta$, ◇) as a function of Tween 20 concentration (μM) for mixtures of 0.1% BSA and Tween 20 adsorbed on water. Angular frequency = $0.81 \text{ rad}\cdot\text{s}^{-1}$. $T = 20^\circ\text{C}$. Adsorption time = 60 min. The arrows indicate the cmc of Tween 20 (Rodríguez Niño, 1997).

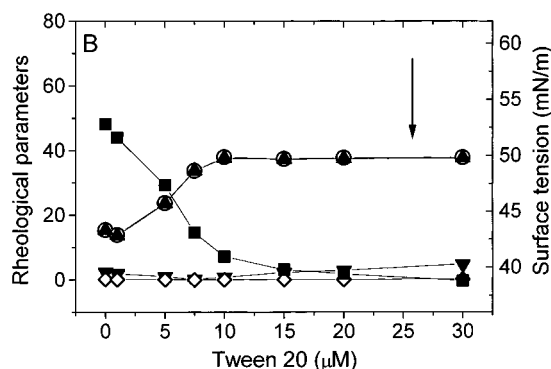


Figure 8. Surface tension (σ , ■, mN/m), surface dilational modulus (E , ○, mN/m), elastic component (E_d , ▲, mN/m), viscous component ($\eta_d \omega$, ▼, mN/m), and loss angle tangent ($\tan \theta$, ◇) as a function of Tween 20 concentration (μM) for mixtures of 0.1% BSA and Tween 20 adsorbed on a 1 M ethanol aqueous solution. Angular frequency = $0.81 \text{ rad}\cdot\text{s}^{-1}$. $T = 20^\circ\text{C}$. Adsorption time = 60 min. The arrows indicate the cmc of Tween 20 (Rodríguez Niño, 1997).

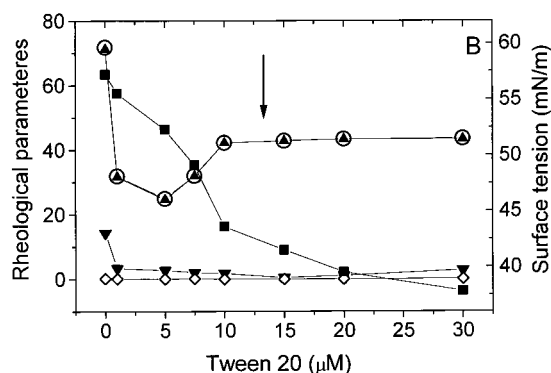


Figure 9. Surface tension (σ , ■, mN/m), surface dilational modulus (E , ○, mN/m), elastic component (E_d , ▲, mN/m), viscous component ($\eta_d \omega$, ▼, mN/m), and loss angle tangent ($\tan \theta$, ◇) as a function of Tween 20 concentration (μM) for mixtures of 0.1% BSA and Tween 20 adsorbed on a 0.5 M sucrose aqueous solution. Angular frequency = $0.81 \text{ rad}\cdot\text{s}^{-1}$. $T = 20^\circ\text{C}$. Adsorption time = 60 min. The arrows indicate the cmc of Tween 20 (Rodríguez Niño, 1997).

dilational modulus decreased to a minimum at Tween 20 concentration of $5 \mu\text{M}$ and then increased and returned to a plateau value at $10 \mu\text{M}$ Tween 20. The plateau value of the surface dilational modulus for

BSA–Tween 20 mixed films was highest on water and decreased for sucrose and then ethanol.

The results obtained in this work supported and strengthened the idea that the superficial behavior of mixed emulsifiers depends on the emulsifier mixture and the subphase compositions (Rodríguez Niño et al., 1997; Wilde et al., 1997). In fact, the surface rheological properties are maximal for pure BSA films in water and aqueous solutions of sucrose. The minimum observed in mixed films at Tween 20 concentration of $5\text{--}7.5 \mu\text{M}$ could be associated with a transition in the adsorbed layer structure due to protein–surfactant interactions. Therefore, it can be concluded that at higher protein/surfactant ratio in mixed films, the protein–protein interactions are predominant, which agrees with a higher surface dilational modulus value (Figures 7–9). At higher surfactant/protein ratio, the surfactant–surfactant interactions are predominant, but these interactions are weaker than protein–protein interactions, which agrees with a lower superficial dilational modulus at the plateau value (Figures 7–9). The minimum value of the surface dilational modulus occurs at a Tween 20 concentration of the same order of magnitude but lower than the cmc (see arrows in Figures 7–9). At this Tween 20 concentration, BSA and Tween 20 as single molecules compete at the interface. This protein/surfactant ratio is a singular composition in mixed films with specific properties—such as structure of the adsorbed layer (Clark et al., 1993; Courthaudon et al., 1991) and drainage and superficial diffusion in thin films (Clark et al., 1990a,b; Bos et al., 1967; Sarker et al., 1995; Wilde et al., 1997)—that in turn affects the stability of foams and emulsions (Chen and Dickinson, 1995a–c; Sarker et al., 1995).

The differences observed with BSA–Tween 20 mixed films adsorbed on ethanol aqueous solutions could be due to the existence of competitive adsorption of ethanol, BSA, and Tween 20 at the interface—as discussed previously from a kinetic point of view—and to the fact that interactions of ethanol with emulsifiers, especially with protein, at both the interface and the bulk phase (Tanford, 1962; Brands and Hunt, 1967) could exist. As a consequence of these interactions with ethanol, protein–protein and protein–surfactant interactions tend to diminish, which agrees with the observed lower plateau surface dilational modulus (Figure 8) than that on water (Figure 7) or sucrose aqueous solution (Figure 9). The existence of protein–surfactant interactions in the presence of ethanol can be proved by the positive synergistic effect of Tween 20 on the surface dilational modulus of pure BSA films. That is, the weaker protein–protein interactions as a consequence of the denaturing effect of ethanol (Rodríguez Niño et al., 1997a) could facilitate the existence of protein–surfactant interactions at the interface and in turn could increase the film viscoelasticity, as the Tween 20 concentration increases, which agrees with data presented in Figure 8.

ABBREVIATIONS USED

A = area of surface element (m^2); E = surface dilational modulus (mN/m); E_d = storage or elastic component of the modulus (mN/m); E_v = loss or viscous component of the modulus (mN/m); k = first-order rate constant (min^{-1}); η_d = surface dilational viscosity ($\text{mN}\cdot\text{s}/\text{m}$); π = surface pressure (mN/m), π_∞ , π_0 , and π_θ , surface pressure at steady state, at time $\theta = 0$, and at any time,

respectively (mN/m); φ = phase angle (deg); θ = time (min); σ = surface tension (mN/m); σ_0 = subphase surface tension (mN/m); σ_∞ = surface tension at equilibrium (mN/m); ω = angular frequency (rad·s⁻¹).

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