Effect of Ethanol on Monoglyceride Monolayers at the Air–Aqueous Phase Interface

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ABSTRACT: We have studied the behavior of monopalmitin and monoolein monolayers, spread at the air-aqueous phase interface, as a function of temperature and surface pressure. The subphases were aqueous ethanol solutions at 0.5 and 1 mol/L. The structural characteristics of these films at interface were deduced from the π-A isotherms, as measured with an automated Langmuir-type film balance. The monolayer structure and stability were functions of hydrocarbon chain length and the presence of a double bond. Generally, the factors that decreased monolayer stability produced transformations toward configurations with more expanded structures. Changes in the subphase composition had a direct influence on the monolayer molecular structure. This study showed the existence of interactions between film and ethanol molecules at the interface. As a consequence of these interactions, a contraction in the monolayer structure was observed. The magnitude of interactions between monoglyceride and ethanol molecules at the interface depends on the surface pressure, temperature, and surface composition. Stronger film-substrate interactions produced changes in monolayer stability. Relationships between film elasticity and structural characteristics are also discussed.

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KEY WORDS: Air–water interface, ethanol, film balance, film elasticity, monoglyceride, monolayer, monolayer structure, monoolein, monopalmitin, surface pressure.

Emulsifiers (proteins and low-molecular-weight emulsifiers) are employed in a wide variety of applications within the food industry. In particular, they are essential in the manufacture of many food colloids, such as margarine, low-fat spreads, ice cream and salad dressings. Understanding how the emulsifiers interact at the oil–water and air–water interfaces and how they behave under different environmental conditions is of considerable practical interest for improving the formation and stabilization of food emulsions and foams (1).

Many food emulsions contain a mixture of a macromolecular emulsifier (proteins) and low-molecular-weight emulsifiers, usually a fatty acid derivative. In oil-in-water emulsions, adsorbed protein at the oil–water interface provides the principal mechanism whereby emulsion droplets are stabilized against flocculation and coalescence. Less clear-cut, however, is the stabilizing role of the low-molecular-weight emulsifiers (2).

Major components of foods are solutes that have significant effects on their physicochemical and functional properties. In previous studies, we showed that the aqueous phase composition has an important impact on the lipid–lipid and lipid–subphase interactions and, as a consequence, on the structure and stability that films of these emulsifiers present at the air–water interface (3–10). These effects have direct consequences on the characteristics and properties of final dispersed products (emulsions and foams).

This work is an extension of studies of mono- and diglyceride monolayers, spread at the air-aqueous phase interface (3–10), that are of interest for food formulations. This paper is concerned with the role of some low-molecular-weight emulsifiers (monopalmitin and monoolein) spread on the air–water interface in aqueous systems containing ethanol. The addition of ethanol in the aqueous phase is of practical importance in the manufacture of alcoholic beverages such as cream liqueur and beer (1,11). The incorporation of ethanol in aqueous protein solutions improves foam stability and foam capacity due to the reduction in bubble size by means of a decrease in the surface tension of the aqueous solution (12–14). At the same time, the ethanol molecules may be adsorbed at the air-water interface and take part in interactions with the protein molecules (15), producing changes in the protein conformation at the interface or even protein denaturation (16,17), which correlates with poor foaming properties (1). However, the addition of ethanol in the aqueous phase produced more condensed and unstable monoolein monolayers (18).

EXPERIMENTAL PROCEDURES

Monopalmitin (1-monohexadecanoyl-*rac*-glycerol) and monoolein [1-mono-(*cis*-9-octadecenoyl)-*rac*-glycerol], more than 99% pure, were acquired from Sigma (St. Louis, MO) and dissolved in a 9:1 (vol/vol) hexane and ethanol mixture. Analytical-grade hexane and ethanol were obtained from Merck (Darmstadt, Germany) and used without further purification. Water was purified with a Milli-Q apparatus (Millipore, Milford, MA) and used to prepare the substrate solution. To establish the ethanol influence on monoglyceride films, aque-

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*To whom correspondence should be addressed at Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, c/o Prof. García González, s/n 41012, Sevilla, Spain. E-mail: jmrodri@cica.es ous solutions with ethanol contents at 0.5 and 1 mol/L, no more than 1 d old, were used. The absence of surface-active contaminants in the water and in the hexane-ethanol mixture was verified by measuring the surface pressure in the entire area interval in the absence of amphiphilic substances.

Measurements of surface pressure, π, vs. average area per molecule, *A*, were performed on a fully automated, Langmuir-type film balance (Lauda, Postfach, Germany). The apparatus, techniques, and experimental conditions used to study π-*A* isotherms on aqueous solutions have been described in detail elsewhere (4–7). The π-*A* isotherms were registered at temperatures of 5, 20, 25, 30, 35, and 37.5 (or 40) °C.

Precautions were taken in the collection of reliable π-*A* isotherms by the continuous compression methods used in this work. This is especially important for monolayers that are unstable. In previous works performed in this laboratory, we observed that monostearin monolayers spread on aqueous ethanol solutions are unstable (3,5,6). For this reason, the choice of compression rate is important (19,20). In the present work, the compression rate was 6.2×10^{-2} nm^2 · molecule^{-1} · min^{-1}. This value ensures reproducibility in the present work, as was observed previously with other monoglyceride-water systems (6,7,9).

RESULTS AND DISCUSSION

Structural characteristics of monopalmitin films. The π-*A* isotherms of monopalmitin monolayers, spread on the air–water interface (9), will be used as reference for monopalmitin monolayers spread on ethanol aqueous solutions. Figure 1 shows the π-*A* isotherms obtained for monopalmitin monolayers spread on the air–aqueous phase interface at different ethanol concentrations. It is seen that the structural polymorphism is the same for all generally encountered structures of monolayers except the gaseous structure (21). These structures were a function of the aqueous phase composition and temperature. A change toward more expanded monolayer structures was observed as the temperature was increased. These films have the same structural polymorphism in the presence and in the absence of ethanol. However, more condensed films were obtained when ethanol was present in the aqueous phase. In addition, the condensation effect increased with the solute concentration. That is, π-*A* isotherms were displaced toward the π axis with ethanol in the aqueous phase, especially at higher ethanol concentrations. The same effect was observed for monostearin monolayers spread on aqueous ethanol solutions (3,7).

Some complementary conclusions can be deduced from the phase diagram obtained (Fig. 2) from the π-*A* isotherm (Fig. 1). As a consequence of the addition of ethanol to the aqueous phase, we observed that (i) the collapse pressure decreased, especially at higher temperatures and at the higher ethanol concentrations in the aqueous phase, (ii) the surface pressure for the liquid-condensed to solid transition was lower and the region for the liquid-condensed structure was reduced in the presence of ethanol, and (iii) higher temperatures were necessary to obtain a monolayer with liquid-expanded structure when monopalmitin was spread on aqueous solutions of ethanol. The region for this structure was lower

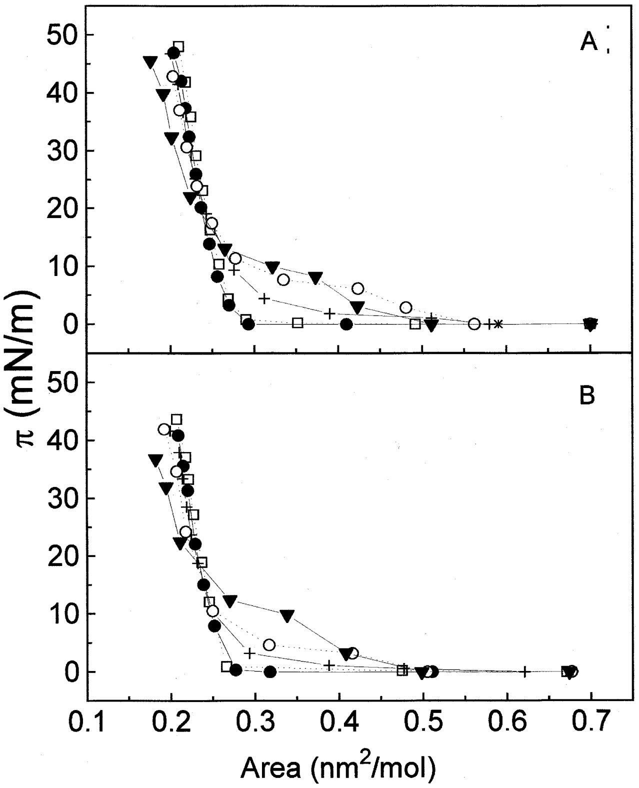

FIG. 1. π-A isotherms for monopalmitin monolayers spread on aqueous solutions of ethanol at (A) 0.5 M and (B) 1 M. Temperatures: (□) 5, (●) 20, (+) 25, (○) 30, (▼) 35°C.

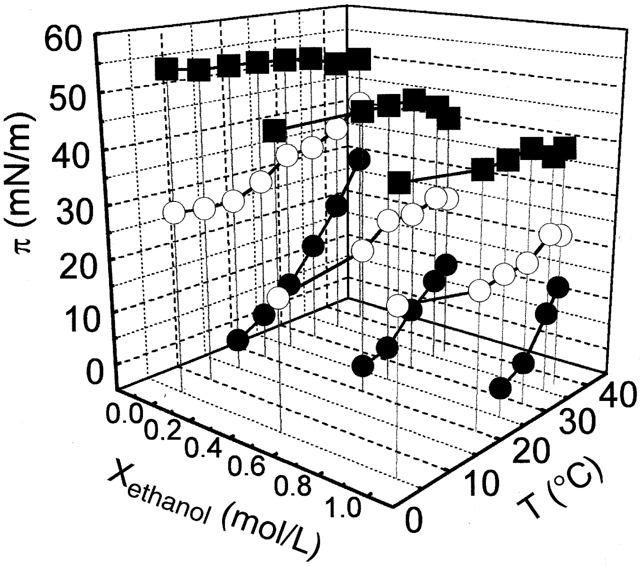

FIG. 2. Phase diagrams (surface pressure for transitions between monolayer structure or at the monolayer collapse vs. temperature) deduced from monopalmitin monolayers spread on aqueous solutions of ethanol. Points corresponding to (■) represent monolayer collapse; (○), solid to liquid-condensed transition; and (●), liquid-condensed to liquid-expanded transition.

in the presence of ethanol in the aqueous phase. However, the ethanol concentration in the aqueous phase did not have a significant influence on these changes.

These results suggest that there was an increase in the lipid-subphase interactions when ethanol was added to the aqueous phase. Molecules of ethanol are amphiphilic, with a short hydrocarbon chain and a polar –OH group. Measurements of the surface tension for aqueous solutions of ethanol as a function of temperature and solute concentration show a decrease in the value for this parameter at higher ethanol concentrations (22). Thus, ethanol tends to be located at the interface between monolayer molecules and can establish van der Waals forces with the hydrophobic chains of surfactants, besides the dipole-dipole interactions with the polar group of monopalmitin. The increase in interactions at the interface produces more condensed films.

As was previously observed with monostearin monolayers (6), ethanol destabilizes monopalmitin monolayers, as was deduced from the displacement of the π-*A* isotherms toward lower molecular areas at higher temperatures and higher surface pressures. An aqueous solution of ethanol at 1 mol/L increased monolayer instability more than less-concentrated ethanol aqueous solutions. Relaxation measurements with monopalmitin films spread on aqueous solutions of ethanol (3) showed that, in fact, there was a loss of molecules from the interface due to their introduction in the adjacent aqueous phase. This process implies an initial molecular dissolution, followed by a simultaneous diffusion and collapse at the higher surface pressures. Film instability in the presence of ethanol agrees with higher interactions between monolayer molecules and between the molecules and ethanol in the subphase.

Values for the molecular area at the beginning of the compression (A_o), and the limiting area (A_{lim}), the molecular area obtained by extrapolation to $\pi = 0$ the solid part in the π-*A* isotherm, were deduced from the π-*A* isotherm. These results are shown in Figure 3. A_o values are reduced when the ethanol concentration increases, at all temperatures, due to film condensation. Nevertheless, the limiting area values are higher in the presence of ethanol only at the lower temperatures, while a significant decrease was observed as temperatures increased. This effect, which was previously also observed with palmitic acid monolayers spread on an aqueous solution of ethanol at 0.5 M (23), can be attributed to the displacement of the π-*A* isotherm toward the π axis, a consequence of the film's instability.

Structural characteristics of monoolein monolayers. Figure 4 shows the π-*A* isotherms for monoolein monolayers spread on aqueous ethanol solutions. Figure 5 shows the phase diagrams deduced from the π-*A* isotherms for monoolein monolayers spread on water (9) and aqueous ethanol solutions, respectively. Only the liquid-expanded structure was observed for monoolein monolayers spread on water and aqueous ethanol solutions. The molecular area interval at which a monolayer exists was reduced in the presence of ethanol in the aqueous phase. The monolayer collapsed at lower surface pressures in ethanol aqueous solutions, especially if the solute concentration was increased. A similar effect was observed as the temperature was increased.

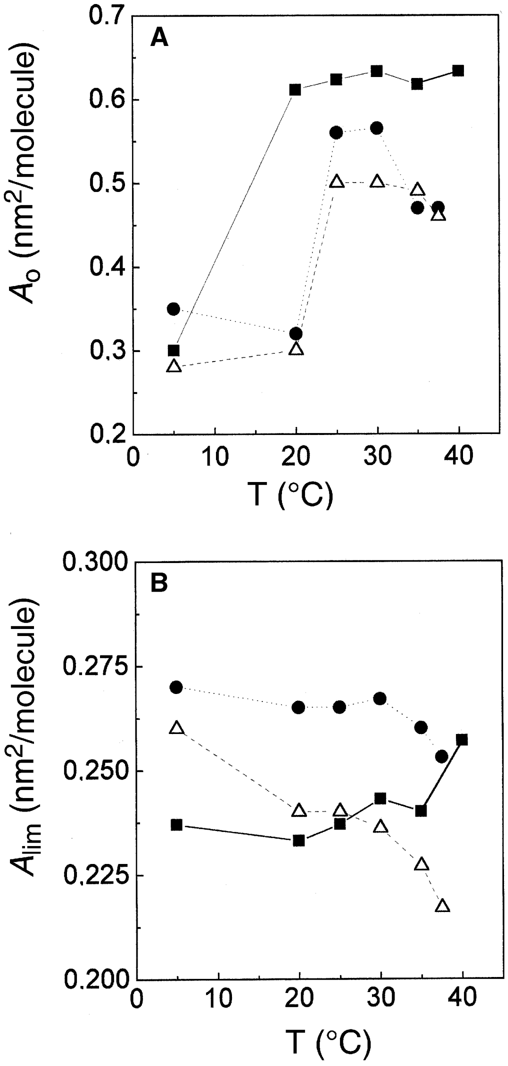

FIG. 3. (A) Molecular area at which the surface pressure begins to be registered, A_o (nm^2/molecule) and (B) limiting area, A_{lim} (nm^2/molecule), as a function of temperature for monopalmitin monolayers spread on (■) water and aqueous solutions of ethanol at (●) 0.5 M and (△) 1 M.

Table 1 shows the values for the molecular area at which the surface pressure began to be registered (A_o). A reduction

TABLE 1
Molecular Area at Which the Surface Pressure Begins to be Registered (A_o)[a]

T (°C)	Water	Ethanol (0.5 M)	Ethanol (1 M)
5	0.75	0.70	0.55
20	0.70	0.59	0.50
25	0.71	0.59	0.50
30	0.71	0.58	0.46
40	0.67	0.58	0.44

[a] Presented as a function of temperature, for monoolein monolayers spread on ethanol aqueous solutions. The units for A_o are nm^2/molecule.

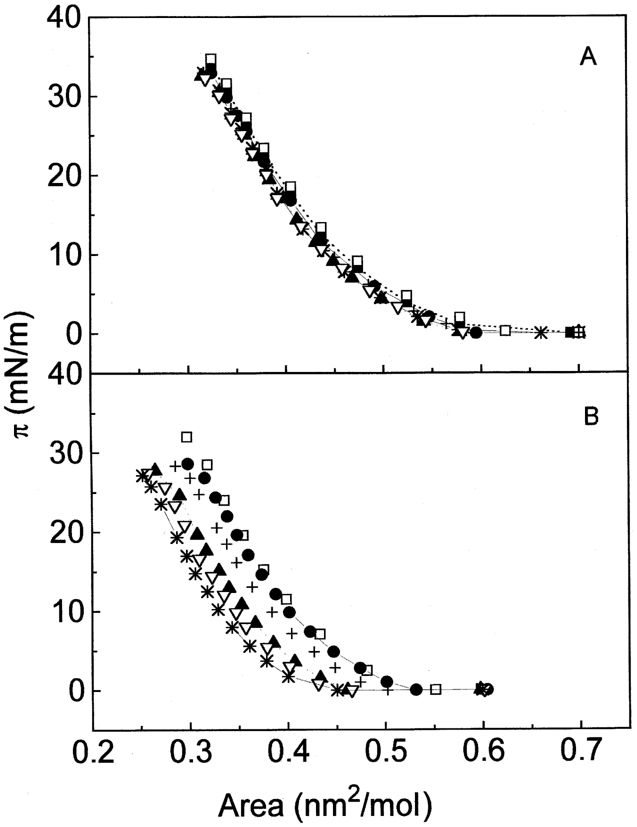

FIG. 4. π-A isotherms for monoolein monolayers spread on aqueous ethanol solutions at (A) 0.5 M and (B) 1 M. Temperatures: (□) 5, (■) 10, (●) 20, (+) 25, (▲) 30, (▽) 35, (✳) 40°C.

in the value for this parameter in the presence of ethanol is consistent with film condensation. In fact, a higher compression level of the film was necessary to measure an initial surface pressure, more so for the aqueous solution of ethanol at 1 M.

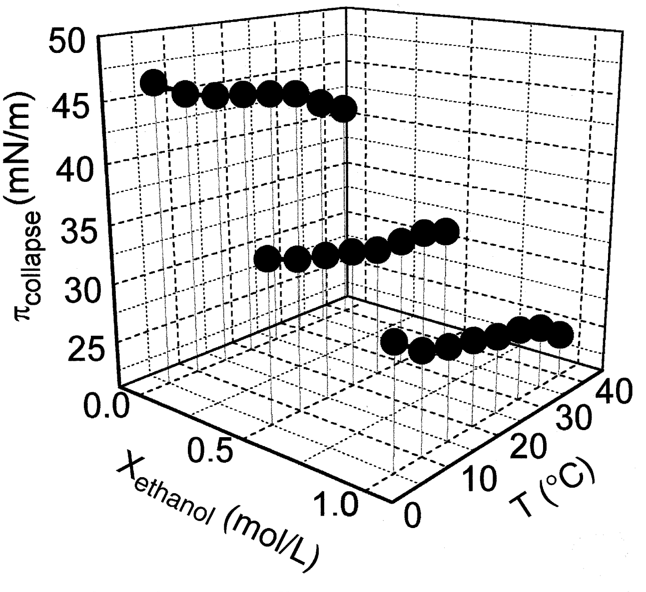

FIG. 5. Phase diagrams (collapse pressures vs. temperature) for monoolein monolayers spread on aqueous solutions of ethanol.

Film elasticity. Film elasticity, expressed by the modulus $-d\pi/dA$, the slope of the π-A isotherm at a specific surface area, is a useful parameter to describe the relationship between structure and monolayer stability. It was previously established that film stability is a phenomenon related to the cohesive forces in the film and to interactions in the subsurface region (4). Film elasticity values quantify the intermolecular and film subphase interactions. Elasticity is a measurement of the resistance to change in the film area (24,25). Highly cohesive interfacial films are more resistant to mechanical deformation. Besides, dynamic surface pressure and elasticity play an important role in many processes, such as emulsification, foam formation, extraction, distillation, or chemical surface reactions (26,27).

The elasticity modulus of monopalmitin and monoolein monolayers spread on water and aqueous solutions of ethanol are shown in Figure 6. Different values can be observed for the same lipid with the same structure, as a function of temperature, surface pressure, and subphase composition. Thus, the mechanical properties of the films give a complementary description of monolayer characteristics.

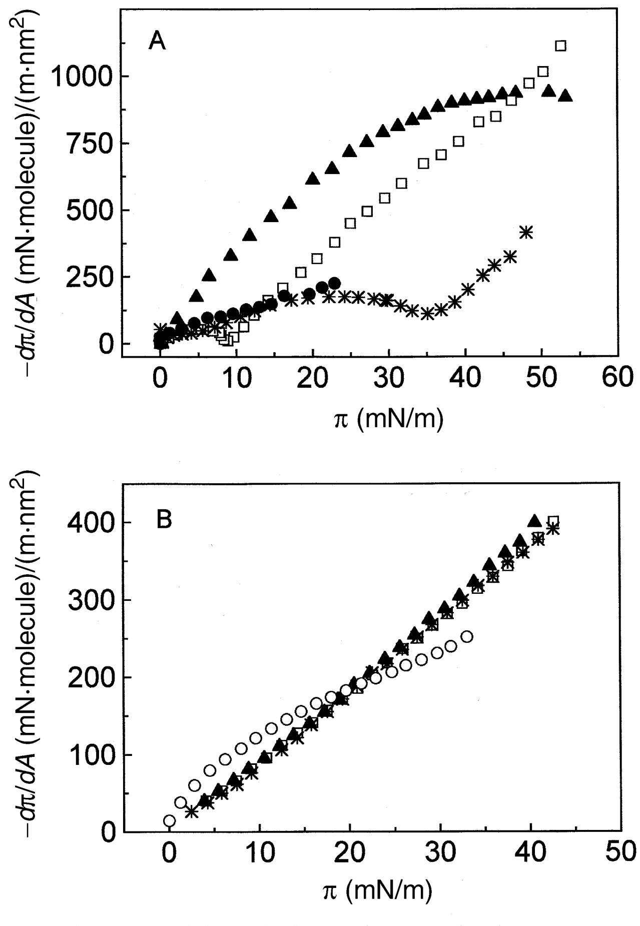

FIG. 6. Elasticity modulus ($-d\pi/dA$) as a function of surface pressure. (A) Monopalmitin monolayers spread on water at 10°C (▲); water at 25°C (□), water at 40°C (✳); and ethanol 0.5M at 25°C (●). (B) Monoolein monolayers spread on water at 10°C (▲); water at 25°C (□), water at 40°C (✳); and ethanol 0.5 M at 25°C (○).

With water in the subphase, the higher values of the elasticity modulus appear at the higher values of surface pressure. The monolayer molecules are more packed, and the interactions between hydrocarbon chains are higher. That is, the monolayer becomes more rigid. An increase in the temperature reduces the values for the film elasticity if the lipid is monopalmitin.

Interactions between monolayer molecules are higher with ethanol in the subphase, so the π-A isotherms appear at lower surface areas, and the elasticity modulus is greater for the same conditions of surface pressure and temperature. Similar effects were found for monostearin films (6). The elasticity moduli of monopalmitin and monoolein monolayers, in the presence or absence of ethanol, are in concordance with the structures previously established for these systems (28).

Monopalmitin and monoolein are monoglycerides with important structural differences, due to differences in the hydrocarbon chain. Monopalmitin has a saturated hydrocarbon chain with 16 carbon atoms, which tends toward a vertical orientation at the air–water interface with close packing at the higher surface pressures. If the space between film molecules is large, this chain lies over the interface. However, for monoolein molecules, close packing among hydrocarbon portions is not possible, even when the film is highly compressed, due to the existence of a double bond between the C_9 and C_{10} atoms. So, at any aqueous subphase solution, monoolein molecules adopt a liquid-expanded structure as the monolayer is compressed.

We have studied the structural characteristics of monomolecular films of monopalmitin and monoolein (two lipids used as food emulsifiers) on the air–aqueous ethanol solution interface as a function of temperature and solute concentration. Interactions between monoglyceride monolayer molecules can be affected by ethanol in the subphase. Ethanol acts as a surfactant and is able to adsorb at the interface. For more expanded monolayers, adsorption of the ethanol molecules at the interface is enhanced. Thus, hydrophobic interactions between hydrocarbon chains as well as hydrophilic interactions between head groups or intermolecular hydrogen bonding between ethanol and monopalmitin or monoolein are possible. As a consequence of these interactions, more condensed and unstable monolayers are produced. Figure 7 shows a model of the molecular arrangements at the interfacial region in the presence of ethanol. The interactions between the monoglyceride molecules at the interface and ethanol in the aqueous phase can give rise to an attraction of the former toward the aqueous bulk phase. When the polar group of monoglyceride molecules submerges, the hydrocarbon chains also do so, partially reducing the area occupied by the molecules, in agreement with the condensation effect found in this work for ethanol in the subphase on the structure of monoglyceride monolayers.

From the results of this investigation and previous studies (3–10,18,22,29), it may be inferred that interfacial composition is not the only parameter that can influence the properties of mono- and diglyceride monolayers at the air–aqueous phase interface. These studies have highlighted significant differences between interfacial characteristics of mono- and diglycerides. There is experimental evidence that the interfacial characteristics of insoluble polar lipids are determined by the lipid and the aqueous-phase composition (water and aqueous solutions of ethanol, glycerol, sugars, electrolytes, and pH).

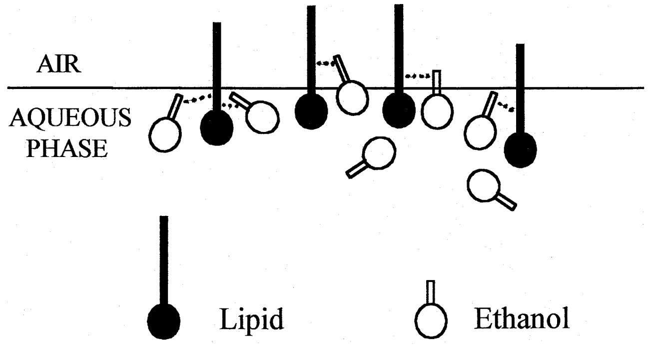

FIG. 7. Molecular model for monoglyceride monolayers at the air-aqueous ethanol phase interface.

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