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Relaxation phenomena in phospholipid monolayers at the air–water interface

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Abstract

In this work we are concerned with the study of long-term relaxation phenomena in dipalmitoyl phosphatidylcholine (DPPC) and dioleoyl phosphatidylcholine (DOPC) monolayers spread at the air–water interface as a function of the surface pressure and the aqueous phase pH (pH 5, 7, and 9). Long-term relaxation phenomena were determined in an automated Langmuir-type film balance at constant temperature (20 °C). Two kinds of experiments were performed to analyze relaxation mechanisms. In one, the surface pressure (π) was kept constant, and the area (A) was measured as a function of time (θ). In the second, the area was kept constant at monolayer collapse and the surface pressure was decreased. This decrease was measured as a function of time. Various relaxation mechanisms, including monolayer molecular loss by dissolution, collapse, and/or organization/reorganization changes, can be fitted to the results derived from these experiments. These relaxation mechanisms are pH and phospholipid dependent. In the discussion, special attention will be given to the effect of the relaxation phenomena on the hysteresis in π–A isotherms before and after the relaxation experiment. At π lower than the equilibrium spreading pressure (π_e) the relaxation phenomena are mainly due to the loss of DPPC or DOPC molecules by desorption into the bulk aqueous phase. The formation of interfacial macroscopic vesicles, which are dissolved into the bulk phase, makes the phospholipid monolayer molecular loss irreversible. At the collapse point (at $\pi > \pi_e$), the relaxation phenomena may be due either to collapse for DPPC and/or to a complex mechanism including competition between desorption and monolayer collapse for DOPC.

Keywords: Food emulsifier; Phospholipid; Air–water interface; Interfacial dynamic properties; Relaxation phenomena; Collapse; Desorption

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1. Introduction

Food dispersions (emulsions and foams) are complicated formulations containing many emulsifiers, which may show surface activity by themselves (biomacromolecules and low-molecular-weight emulsifiers) or by associating with each other [1–4]. Emulsifier molecules comprise both hydrophilic and hydrophobic portions; this gives rise to their surface activity. In order to control the production, stability, and other organoleptic and textural properties of food dispersions, detailed knowledge of the role of emulsifiers at fluid interfaces is required.

The highly complex mechanisms involved in the formation and stabilization/destabilization of food dispersions make fundamental studies in applied systems difficult. Thus, the study of characteristics of food emulsifier monolayers at the air–water interface as a food model presents several advantages [5–7]. An understanding of the structure and dynamic properties of the monolayer is essential for the prediction of the properties of food colloids stabilized by emulsifiers. This is because the formation, stabilization, and destabilization of foams and emulsions are governed to a large extent by the dynamic characteristics of the film around the bubbles or the droplets [8,9]. The dynamics of liquid interfaces comprise adsorption kinetics, interfacial relaxation, and mechanical (rheological) behavior [10–13]. There are data in the literature suggesting that surfactant molecules especially are subjected to strong interfacial interaction [12–16]. Moreover, molecular orientational changes and interfacial aggregations can significantly modify the relaxation behavior of surfactants at fluid–fluid interfaces [17,18]. The effect of the bulk phases also has to be discovered. The interaction of surfactant with solvent molecules may cause different structures and complicated relaxation phenomena [10–13].

The aim of this work is the study of relaxation phenomena in phospholipid (DPPC and DOPC) monolayers spread at the

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air–water interface as a function of processing variables (surface composition and surface pressure) and aqueous phase pH. In previous works we have analyzed the relaxation phenomena in pure monoglycerides [19], proteins [20], and their mixtures [21] at the air–water interface. Non-equilibrium processes occurring in systems containing fluid–fluid interfaces with a surfactant present are of practical importance [22–25].

Phospholipids are ionizable low-molecular-weight emulsifiers, which are needed to increase colloidal stability and provide interfacial interactions between food components [26–28]. Such interactions are important factors in obtaining emulsion stability, foam formation, and increased shelf life in many foods. The main applications of phospholipids in foods are in traditional foods (such as bakery, confectionery or meat products, ice cream, dressings, etc.) or new formulations (low fat and instant foods, high- or low-alcohol food formulations, functional foods, etc.). Thus, the modern production of industrial foods requires a complete understanding of the behavior of emulsifiers used as processing aids, both at interface and in the bulk phases, at equilibrium and under dynamic conditions.

2. Experimental

2.1. Materials

L-α Dipalmitoyl phosphatidylcholine (DPPC) and dioleoyl phosphatidylcholine (DOPC) were supplied by Sigma (>99%). To form the surface film, the phospholipid was spread in the form of a solution, using chloroform/ethanol (4:1, v/v) as a spreading solvent. Phospholipid solutions were prepared every week, divided into small fractions, and stored at 4 °C in order to avoid solvent evaporation and to maintain the concentration of phospholipid. Analytical grade chloroform (Sigma, 99%) and ethanol (Merck, >99.8%) were used without further purification. The water used as subphase was purified by means of a Millipore filtration device (Milllipore, Milli Q^{TM}). To adjust subphase pH, buffer solutions were used. Acetic acid/sodium acetate aqueous solution (CH_3COOH/CH_3COONa) was used to achieve pH 5, and a commercial buffer solution called trizma ($(CH_2OH)_3CNH_2/(CH_2OH)_3CNH_3Cl$) for pH 7 and 9. All these products were supplied by Sigma (>99.5%). Ionic strength was 0.05 M in all the experiments.

2.2. Methods

2.2.1. Surface film balance

Measurements of the surface pressure (π) versus average area per molecule (A) were performed on fully automated Langmuir-type film balance, as described elsewhere [29,30]. The subphase temperature was controlled at 20 °C by water circulation from a thermostat, within an error range of ±0.3 °C. Aliquots of chloroform/ethanol (4:1, v/v) solutions of phospholipid (0.28 mg/mL) were spread on the interface. To allow for spreading of the phospholipid 30 min were allowed to elapse before measurements were taken. The compression rate was 3.3 cm min^{-1}, which is the highest value for which isotherms had been found to be reproducible in preliminary experiments [31]. Each isotherm was measured several times using four new aliquots. The mean deviation was within ±0.3 mN/m for surface pressure and $\pm 0.125 \times 10^{-3}$ m^2/mg for area. All isotherms were recorded continuously by a device connected to the film balance and then analyzed off-line.

Table 1
Equilibrium spreading pressure (π_e) for DPPC and DOPC monolayers spread at the air–water interface at 20 °C and at an ionic strength of 0.05 M

	pH		
	5	7	9
DPPC	49.0	47.0	46.0
DOPC	46.1	46.1	46.1

Two kinds of experiment have been used for the analysis of relaxation in phospholipid monolayers [17]. First, the surface pressure (π) is kept constant, and the area A is measured as a function of time. The results of relative area relaxation A/A_0 versus time were transformed into N/N_0, where A and A_0 are the molecular area at time θ and at the initial moment, respectively, and N and N_0 are, respectively, the number of phospholipid molecules in the monolayer that remain on the interface at time θ and at the initial moment [19]. In the second type of experiment, the area is kept constant (at the collapse) and the surface pressure decreases. This decrease is measured as a function of time.

2.2.2. Data analysis

Various relaxation mechanisms – including desorption, collapse, evaporation, surface chemical reaction, polar group hydration, conformation–organization changes, Marangoni effect, etc. – can be fitted to the results derived from the above-mentioned experiments. Relaxation mechanisms other than desorption and collapse are difficult to quantify in typical experimental relaxation experiments such as those used in this work. Thus, the results were tested by the equations derived for desorption and/or monolayer collapse.

The equilibrium spreading pressure (π_e) is a key parameter for the analysis of the mechanisms that trigger the relaxation phenomena in spread monolayers at the air–water interface [17]. The equilibrium spreading pressure is the maximum surface pressure to which a spread monolayer may be compressed before monolayer collapse. Thus, instability due to monolayer collapse must be rejected at surface pressures lower than π_e. However, the monolayer can be unstable at $\pi < \pi_e$ due to relaxation mechanism other than monolayer collapse, as has been observed with monoglyceride monolayers spread on the air–aqueous phase interface [19]. The values of π_e for the phospholipids studied in this work are included in Table 1 [31].

Desorption of spread monolayers at any constant surface pressure involves two stages [32]. The first is dissolution into the bulk aqueous phase to form a saturated aqueous layer. During the initial non-steady-state period of desorption, the rate of monolayer molecular loss can be expressed by equation (1). The second stage occurs when, after a time, the concentration gradient within the diffusion layer becomes constant and desorption