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Hydration and removal of supported phospholipid films in aqueous surfactant solutions

M.T. Colberg[a], K. Carnes[a], A.E. Sáez[a], C.S. Grant[a,*], K. Hutchinson[b], D. Hesterberg[b]

[a]*Department of Chemical Engineering, North Carolina State University, P.O. Box 7905, Raleigh, NC 27695-7905, USA*
[b]*Department of Soil Science, North Carolina State University, P.O. Box 7619, Raleigh, NC 27695-7619, USA*

Abstract

Dynamic studies of the hydration and removal of phospholipid films attached to solid substrates were performed. The phospholipids used were 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine (DPPE), and a modification of DPPE containing a fluorescent molecular probe: *n*-(5-fluoresceinthicarbamoyl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, triethylammonium salt (FITC-DPPE). The phospholipid films were exposed to water and aqueous solutions of the anionic surfactant sodium dodecyl sulfate (SDS). The film mass was determined as a function of time by means of a quartz crystal microbalance (QCM). The crystalline structure of the film during the hydration process was analyzed by means of wide-angle X-ray diffraction. At low surfactant concentrations (below 20% of the critical micelle concentration (CMC)), the presence of surfactant increased the hydration rate of the film, as well as its maximum water uptake. At surfactant concentrations as low as 50% of the CMC, competitive hydration and removal of the phospholipid film were observed. X-Ray diffraction measurements show that the crystal structure of the DPPE films did not change significantly upon exposure to water and surfactant solutions. In contrast, FITC-DPPE films exhibited changes in the long-range spacing of their crystalline structure upon hydration.

Keywords: Phospholipid; Quartz crystal microbalance; Hydration; Surfactant

1. Introduction

The hydration of phospholipid films in aqueous environments has been a subject of interest because it is believed to stabilize bilayer structures [1], and may be an essential property in the fusion and endocytosis of biological membranes [2]. The swelling of phospholipid membranes is accompanied by changes in the crystalline structure of the phospholipid [3] when the temperature is greater than the main phase transition temperature (T_c). Under these conditions, relatively high levels of hydration are observed. At temperatures below T_c, hydration still occurs but the lamellar structure of the phospholipid is preserved.

In a previous work [4], we studied the hydration behavior of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine (DPPE) and DPPE with a fluorescent label covalently bound to the molecule: fluorescein isothiocyanate (FITC-DPPE) at 25°C, which is well below the main phase transition temperature for DPPE (T_c = 63°C). That work emphasized measurement of hydration rates of films attached to a solid substrate in the presence of pure water. A quartz crystal microbalance was used to determine the mass of film as a function of time. We also performed X-ray diffraction measurements to determine film structure. Total internal reflection fluorescence (TIRF) experiments were also conducted to explore hydration of the supported films upon exposure to deionized water. We showed that the hydration process of DPPE could be analyzed by means of a simple diffusion model, in which partition rates of water into the film are limited by its molecular diffusion in the solid phospholipid. On the other hand, FITC-DPPE films exhibited a hydration capability that was much higher than that of DPPE films.

In this work we extend our previous hydration studies to the case of phospholipid films exposed to solutions of the anionic surfactant sodium dodecyl sulfate (SDS). The main objectives are to analyze the kinetics of hydration and removal, as well as to determine the evolution of film structure during the process.

* Corresponding author. Tel.: +1 919 5152317; fax: +1 919 5153465; e-mail: grant@eos.ncsu.edu

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2. Experimental

2.1. Materials

The phospholipids used in the experiments were obtained in powder form from Sigma Chemicals, and were stored in a desiccator at temperatures below –20°C. Their purity was above 99.9%. Stock solutions were prepared by dissolving 10 mg of phospholipid in 5 ml of a 9:1 v/v chloroform/methanol solution. The phospholipid concentration in the stock solution was 2 mg/cm^3. Both phospholipids (DPPE and FITC-DPPE, Fig. 1) are insoluble in water, but the fluorescent probe makes the FITC-DPPE molecule more hydrophilic than the DPPE molecule. FITC-DPPE has been used as a fluorescent tracer in phospholipid research and also as a model fluorescent system to study the relation between fluorescence self quenching and hydration [4]. The surfactant used, SDS, was obtained from Fisher Chemicals in powder form. Its critical micelle concentration is 8.2 mM in water at 25°C. The SDS solutions used in the experiments had concentrations ranging between 10 and 90% of the critical micelle concentration (CMC). This surfactant has been widely used in previous works as a typical anionic amphiphile [5].

2.2. Quartz crystal microbalance experiments

The dynamic hydration and removal of phospholipid films attached to a solid substrate was measured by determining the evolution of the total mass of film deposited on a quartz crystal microbalance (QCM). The balance consisted of a polished At-cut quartz crystal slide. The weighing area had two gold electrodes attached to opposite sides of the quartz slide. An oscillating circuit changes the polarity of the electrodes at the crystal's resonant frequency. Any mass that is rigidly bound to the electrode causes a frequency decrease. Changes in mass are directly proportional to frequency changes. The quartz slide was attached to the side of a reservoir using silicone glue. The frequency shift was measured by means of an Elchema FC-299 frequency meter/generator. The signal was passed through an Elchema DAQ-616 analog/digital converter and data acquisition board mounted in a personal computer.

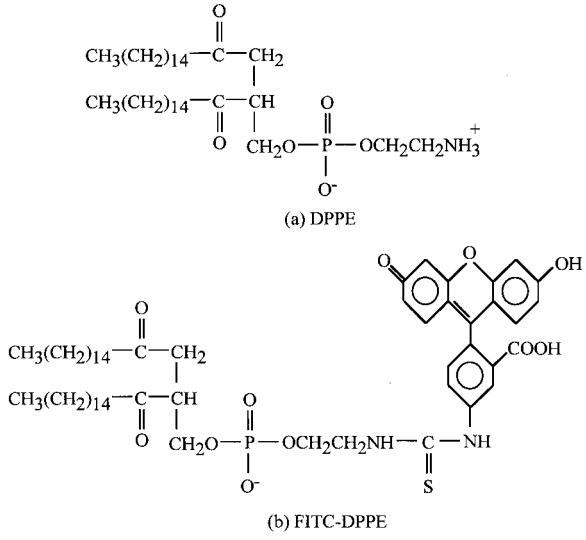


Fig. 1. Molecular structures of the phospholipids used in this work.

The phospholipid films were obtained by depositing 2 μl of a 1 mg/cm^3 phospholipid solution on the electrode. The solution was spread over the electrode and allowed to evaporate at room temperature. The amount of material and solution concentration were selected on the basis of previous studies with similar systems [4,6]. The frequency was measured before and after film deposition to record the mass of dry phospholipid on the balance. The dry mass ranged between 1 and 3 μg. The balance was then immersed in deionized water or surfactant solutions and frequency changes were measured as a function of time. All the experiments were performed at room temperature (25°C).

2.3. X-Ray diffraction experiments

Wide-angle X-ray diffraction was conducted on dry and hydrated DPPE and FITC-DPPE films deposited on quartz slides to determine their crystalline structural properties and structural changes due to the hydration process. A Diano 8500 wide-angle diffractometer was used in these experiments. All measurements were performed at room temperature (25°C). To increase the number of structural layers diffracting X-rays for these experiments, the films were made thicker than those used in the QCM experiments. The quartz slide with the deposited phospholipid film was kept in a desiccator prior to the X-ray diffraction measurement of the dry film. X-Ray diffraction analyses were done with unresolved Cu-K_2 radiation, and all pertinent instrumental parameters, including tube voltage and scanning rate, were kept constant for all experiments. For the measurement of hydrated films the quartz slide was immersed in deionized water or a surfactant solution for a specific period of time, was removed from the liquid and immediately subjected to X-ray diffraction analysis.

3. Results and discussion

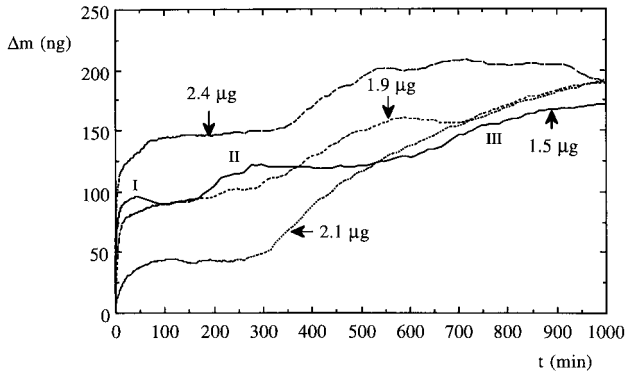
3.1. Hydration of phospholipid films in pure water

Fig. 2 shows the mass change (Δm) of DPPE and FITC-DPPE films as a function of time when exposed to pure water, as measured by the QCM, for different initial masses of dry film. The mass increases in this case are due to water uptake. Therefore, Δm corresponds to the mass of water absorbed by the film.

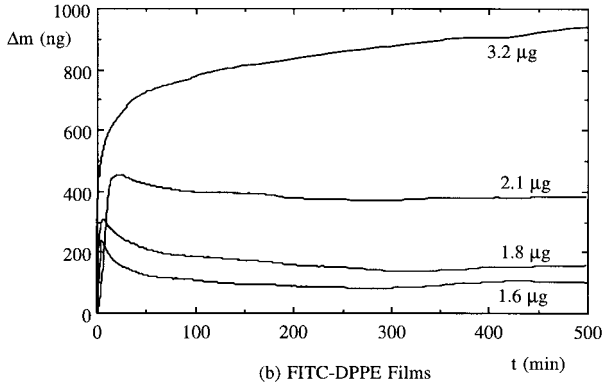
In the experiments with DPPE (Fig. 2a), there is a rapid initial hydration period followed by a stabilization after approximately 30 min. The films then seem to undergo suc-

cessive hydration cycles. Note that the film with phospholipid mass of 1.5 μg exhibits three consecutive hydration cycles (indicated by the numbers adjacent to the curve). The water mass uptake during the first hydration cycle is between 5 and 10% of the dry phospholipid mass, which is consistent with results reported by Wakamatsu et al. [6] in short-term hydration experiments. For this particular case, we have shown in a previous work that the kinetics of the hydration process is controlled by the diffusion rate of water within the film [4]. Our results indicate that after the first cycle there are typically two molecules of water per phospholipid molecule in the hydrated film. This parameter is almost doubled in the final film conformation.

In order to explore possible changes of the DPPE crystalline structure during the hydration process, we performed wide-angle X-ray diffraction experiments on dry and hydrated films (Fig. 3a). The diffraction traces of the dry film show lamellar periodicity up to the 19th order with an average first order d-spacing of 5.53 nm. This value coincides with previously reported values for DPPE (5.52 nm) [3,6]. This suggests that the DPPE films exhibit a multilayer lamellar structure. X-Ray diffraction patterns obtained after exposure to deionized water for 33 h show that there was essentially no change in the crystalline structure of DPPE upon hydration. This was expected since the hydration temperature was substantially below T_c. The absorbed water molecules seem to occupy interstitial spaces in the DPPE

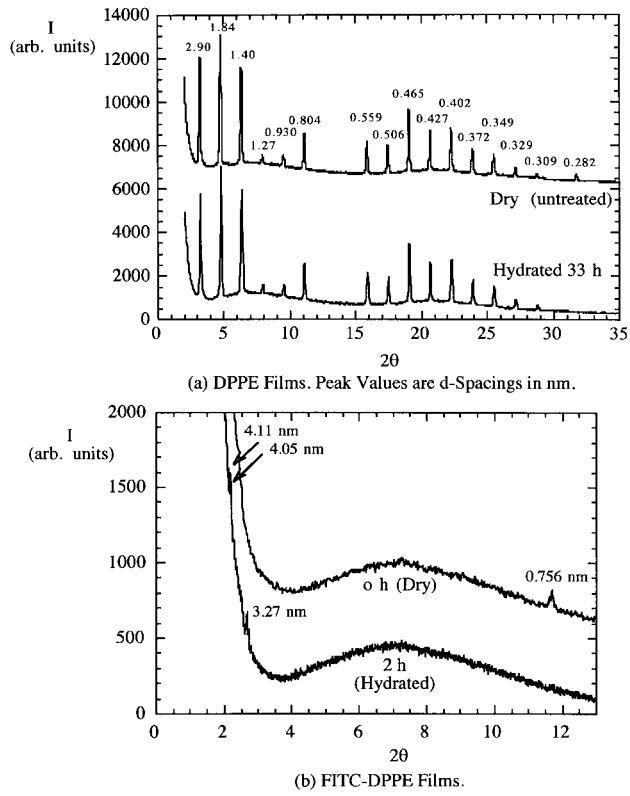


(a) DPPE Films. The Three Consecutive Hydration Cycles of the 1.5 μg Results are Indicated by Roman Numerals.



(b) FITC-DPPE Films

Fig. 2. Hydration of phospholipid films in deionized water. Curve labels indicate the mass of dry film.



(a) DPPE Films. Peak Values are d-Spacings in nm.

(b) FITC-DPPE Films.

Fig. 3. X-ray diffraction patterns of phospholipid films. Hydrated samples were exposed to deionized water for the time indicated.

crystal matrix. Hydration beyond the first cycle (as evidenced by the QCM results) might be a consequence of the formation of water pockets within the film, i.e. there might be phase separation between water and lamellar phospholipid structures, leading to an increase in the water uptake.

Fig. 2b shows the results of hydration of FITC-DPPE films in pure water. The pattern of water uptake is different from that observed in the DPPE films. The film reaches a maximum hydration limit after which the water mass decreases until it reaches an asymptotic value. This behavior is obtained in all films except the thicker film (which corresponds to an initial mass of 3.2 μg of FITC-DPPE). It is interesting to note that the higher the initial mass, the less sharp the peak in water uptake. It is important to emphasize that drying the film after the hydration experiments showed no significant loss in phospholipid mass. This means that the decrease in mass after the maxima is not due to phospholipid losses. In general, FITC-DPPE films tend to absorb more water than DPPE films. This is not surprising if one considers that the fluorescent FITC probe in the FITC-DPPE phospholipid molecule is hydrophilic. An interesting observation is that the hydration capability (measured as molecules of water absorbed per phospholipid molecule) increases with initial film thickness, which might be an indication that thicker films do not have a uniform structure as hydration proceeds. If the region of the film close to the wall exhibits a structural pattern that is affected by the

attachment of the phospholipid molecules to the wall, it is possible that far from the wall the structure resembles an unsupported film.

The presence of a maximum in the hydration curves for FITC-DPPE suggests that as water is absorbed, the film structure changes to a new conformation whose equilibrium hydration capacity is lower than that of the original structure. This leads to a competing effect between the hydration of the parts of the film with high water uptake capability and the rejection of water from the parts of the film adopting the new structure. A reduction in hydration capability of phospholipid films caused by spontaneous phase changes has been observed before for 1,2-dipalmitoyl-1-phosphatidylcholine (DPPC) films [7]. In this case, the structure of the phospholipid changed from a gel structure to an orthorhombic crystalline form.

X-Ray diffraction experiments were conducted with FITC-DPPE films to study the structure of the dry films and to analyze possible structural changes upon hydration. The results are shown in Fig. 3b. The dry film exhibits only one weak peak in the scanning angle range analyzed, corresponding to a d-spacing of 0.756 nm. This would be consistent with a liquid crystalline bilayer structure. Once the film is immersed in liquid water, the single diffraction peak progressively decreases until it disappears and new peaks at low angles emerge after approximately 2 h. Notice that after 2 h the QCM studies (Fig. 2b) showed that the thinner films were rejecting water. This result is consistent with changes in film structure as hydration proceeds: the structure becomes progressively more amorphous as water is absorbed, until a new ordered structure appears within the range of diffracting angles employed, whose hydration capability is lower than the original structure (as revealed by the QCM results). The new structure formed is characterized by d-spacings that are larger than the original structure.

3.2. Behavior of phospholipid films in surfactant solutions

The evolution of the mass of DPPE films in the presence of SDS solutions is presented in Fig. 4. At very low surfactant concentrations (10% of the CMC), the mass uptake of the film increases substantially with respect to the hydration observed in pure water. This mass increase might be due to the partitioning of surfactant into the DPPE film. In terms of total mass uptake, surfactant partitioning competes with water uptake. In order to test this hypothesis, we will assume that the difference between the water and 10% CMC curves in Fig. 4a at long times represents the uptake of surfactant by the film; in other words, we assume that hydration water uptake is independent of surfactant partitioning. This value (approximately 600 ng) can only be taken as an order of magnitude estimate of the amount of SDS that partitions into the film, since possible changes in water uptake due to the presence of surfactant are not taken into account. The partition coefficient of surfactants in phospholipid vesicles suspended in an aqueous medium is usually defined as follows [5]: $K = n_{sp}/(n_p c_s)$, where n_{sp}/n_p is the ratio between the number of moles of surfactant (n_{sp}) and phospholipid (n_p) in the phospholipid bilayers, and c_s is the surfactant concentration of the aqueous solution surrounding the phospholipid vesicles. With the data corresponding to 10% CMC we calculate a value $K = 0.8\ \mathrm{mM}^{-1}$. This value is of the same order of magnitude as the partition coefficient of submicellar SDS in phosphatidylcholine reported by de la Maza et al. [5] ($K = 2.8\ \mathrm{mM}^{-1}$). Therefore, at least part of the mass absorbed by the film in the presence of the 10% CMC solution is SDS. With the QCM alone, it is impossible to discriminate between water and surfactant uptake.

X-Ray diffraction measurements were performed on the DPPE film exposed to the 10% and 20% CMC solution of SDS for 2 h. According to the QCM experiments, most of the mass uptake has taken place in the first 2 h (Fig. 4a). The results show no appreciable changes with respect to the X-ray diffraction pattern shown in Fig. 3a, which indicates that the presence of the surfactant in the film does not alter its crystalline structure.

At a surfactant concentration equal to 20% of the CMC, the DPPE film exhibits a rapid mass uptake to a level that is also higher than the one attained by hydration in pure water. This mass uptake is, however, appreciably lower than that corresponding to 10% of the CMC. In this case, surfactant and water partitioning might compete with phospholipid removal due to solubilization. As the surfactant concentration is further increased to 50% of its CMC, an initial

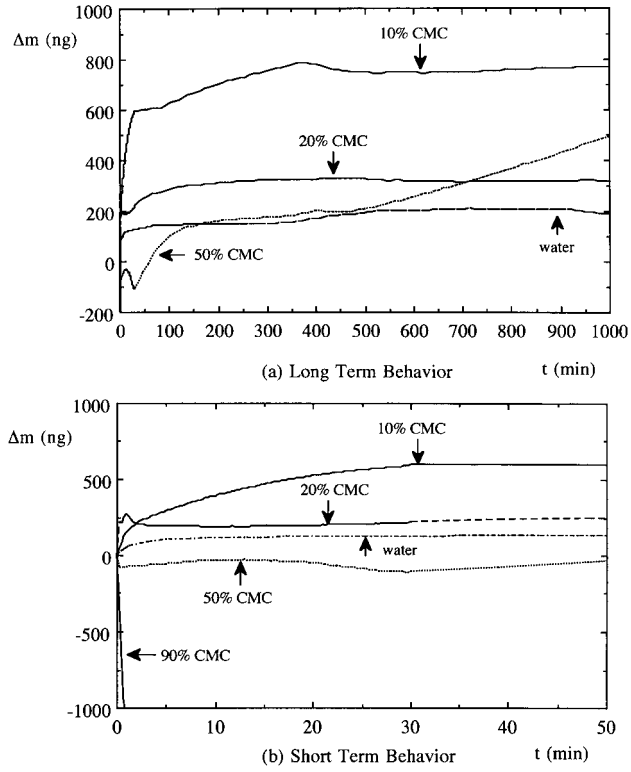


Fig. 4. Behavior of DPPE films in SDS solutions. Film mass: 2.2 ± 0.2 μg. Curve labels indicate surfactant concentration.

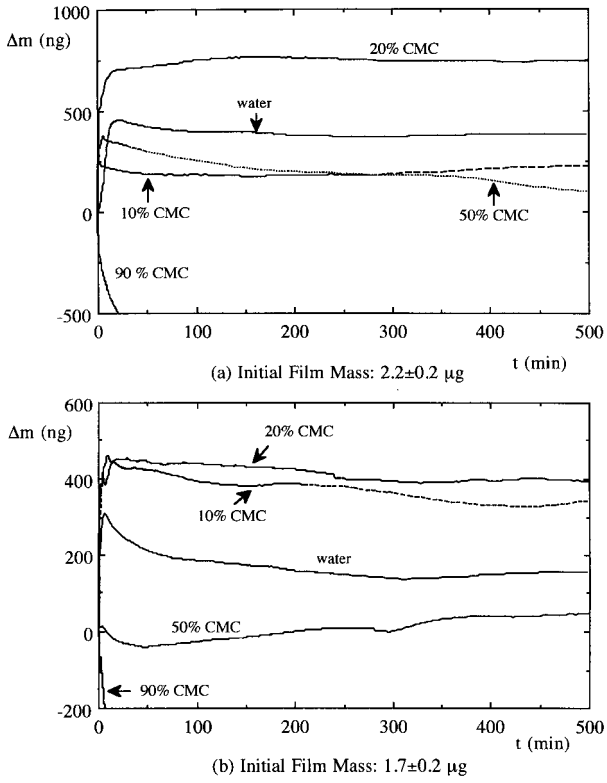


Fig. 5. Behavior of FITC-DPPE films in SDS solutions. Curve labels indicate surfactant concentration.

decrease in mass is observed. In this case, solubilization is faster than the rate of partitioning of surfactant and water into the film. However, the film is not completely solubilized since at long times there is still mass remaining on the microbalance (Fig. 4a). As the CMC is approached, solubilization rates control the evolution of the film's mass. At 90% of the CMC, the film is completely removed from the substrate at a relatively fast rate (Fig. 4b). The fact that complete removal can be achieved at concentrations below the CMC means that mixed micelles can be formed at the interface between the film and the solution even when there are no surfactant micelles in the solution.

The behavior of FITC-DPPE films in SDS solution is presented in Fig. 5. The trends observed in these results are qualitatively similar to the behavior of DPPE films: at low surfactant concentrations, there is a mass increase with respect to hydration in pure water, which is attributed to surfactant partitioning. The exception is the thinner film which shows a lower mass uptake for 10% of the CMC than for pure water. It is interesting to note that film removal is not as appreciable for FITC-DPPE at 20% of the CMC as it was for DPPE. On the other hand, higher surfactant concentrations (50% of the CMC) definitely lead to film removal, and solubilization rates at 90% of the CMC overcome mass uptake rates at lower surfactant concentrations, which is similar to the behavior observed in DPPE films. It is interesting to note that film removal at 90% of the surfactant CMC occurs appreciably faster for the DPPE film than for the FITC-DPPE films, even though DPPE is more hydrophobic than FITC-DPPE. The FITC-DPPE molecule is appreciably larger than the DPPE molecule, a fact that might decrease its propensity to form mixed micelles with the surfactant.

4. Conclusions

The hydration behavior of supported DPPE and FITC-DPPE films in deionized water differs substantially. The more hydrophilic FITC-DPPE exhibits a larger water uptake. X-ray diffraction data showed that the structures of the dry films are different: the DPPE films has a regular lamellar crystalline structure, whereas the FITC-DPPE film exhibits weak long range order. Upon exposure to water and surfactant solutions, DPPE films do not undergo a significant structural change. In contrast, FITC-DPPE films show changes in long-range spacing upon hydration. In the presence of small amounts of surfactant (up to a concentration equal to 20% of the CMC), the phospholipid films exhibit a larger mass increase than in pure water. This is partly due to the partitioning of surfactant molecules into the film. At higher surfactant concentrations, competitive mass uptake and film removal were observed.

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