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An investigation into the effects of surfactants on phospholipid monolayers using a Langmuir-Blodgett film balance

N.M.W. Ah-Fat, D.Q.M. Craig *, K.M.G. Taylor

Centre for Materials Science, School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK

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Abstract

The interactions between egg phosphatidylcholine monolayers and two surface-active agents, sodium lauryl sulphate (SLS) and cetyltrimethylammonium bromide (CTAB), have been studied using a Langmuir-Blodgett film balance using a range of surfactant concentrations below the critical micelle concentration. The presence of SLS in the subphase was shown to result in an increase in the surface pressure (using equivalent surfactant solutions for comparison) while retaining the same basic shape and transitional behaviour of the phospholipid profile. In contrast, the presence of CTAB resulted in a concentration dependent inhibition of film formation. It is concluded that these two surfactants interact with the phospholipid monolayers by different mechanisms over the concentration ranges studied and that surface pressure measurements represent an effective means of characterising such interactions.

Key words: Phospholipid; Monolayer; Surface pressure; Sodium lauryl sulfate; Cetyltrimethylammonium bromide

Surface-active agents (surfactants) are widely used in the pharmaceutical and cosmetic industries but may be toxic to biological membranes. While the mechanisms by which surfactants interact with such membranes are poorly understood, it is believed that at low concentrations of surfactant, the bilayer incorporates amphiphile molecules, resulting in a progressive disruption of the membrane. Eventually, the phospholipid will be solubilized into mixed micelles with the surfactant (Ruiz et al., 1988; Jones, 1992). However, the factors determining the mechanism and extent of incorporation are not understood. A greater knowledge of surfactant-phospholipid interactions would be of considerable pharmaceutical interest, as these materials are incorporated into a number of dosage forms and may also enhance drug absorption from oral and topical preparations. A number of studies have used liposomes as model membranes for detecting and characterising these interactions (e.g., Charaf and Hart, 1991). Furthermore, studies have also been performed in this field using monolayer films as model membranes, including an investigation into the effects of surfactants on cholesterol films (Alexander et al., 1986) and an investigation into the effects of surfactant copolymers on soya phospholipid (Weingarten et al., 1991).

* Corresponding author.

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In this investigation, a Langmuir-Blodgett surface balance has been used to characterise phospholipid films in the presence and absence of surface-active agents in the subphase. This technique involves the measurement of the surface tension of an aqueous surface under different conditions of compression, both in the presence and absence of a surface monolayer. This may be described by Eq. 1, where π is the surface pressure and γ and γ_1 are the surface tensions of the clean surface and the surface containing the monolayer, respectively.

$$\pi = \gamma - \gamma_1 \quad (1)$$

In the apparatus used here, compression takes place via the movement of two beams across the surface. The difference in surface tension at any point is designated the surface pressure and may be related to the area occupied by each molecule within the monolayer. This technique has been used in a number of studies of pharmaceutical interest, including an investigation into the effects of water-insoluble penetration enhancers (azone and oleic acid) on the integrity of phospholipid films (Lewis and Hadgraft, 1990) and a study into the effects of copolymers on phospholipid monolayers in relation to the stability of emulsions (Weingarten et al., 1991). Furthermore, a number of studies have been conducted into the effects of drugs on phospholipid monolayers, including investigations using antihistamines (Attwood and Udeala, 1975), insulin (Birdi, 1976), hydrocortisone (Cleary and Zatz, 1973) and indomethacin (Sicre and Cordoba, 1989). There is therefore considerable potential for extending the use of this technique as a means of characterising the interactions of a number of biologically active molecules with phospholipids, including surface-active agents. In this study, we have investigated the use of the technique as a means of examining the interactions of two commonly used surfactants, sodium lauryl sulphate (SLS) and cetyltrimethylammonium bromide (CTAB) with egg phosphatidylcholine monolayers.

The Langmuir-Blodgett trough (Nima Technology Ltd) comprises a circular PTFE trough with two PTFE barriers which move together or apart in an arc motion, as described by Grunfeld (1993). Surface tension was measured using a Wilhelmy plate, with a piece of chromatography paper being suspended in the aqueous phase between the two arms. Egg phosphatidylcholine (EPC, Merck, Darmstadt), purified by a standard chromatographic method (Bangham et al., 1974), was prepared as a 1 mg/ml solution in chloroform and purified water from an Elgastat UHQ-PS unit was used for the subphase throughout the study. 100 μl of phospholipid solution were placed on the surface in two aliquots using a 50 μl syringe. A compression rate of 8.33 cm^2/s was used throughout. For studies involving surface-active agents in the subphase, SLS (BDH Ltd) and CTAB (BDH Ltd) were used at a range of concentrations below their critical micelle concentrations (CMC: 0.2385 and 0.0299% w/v, respectively). All runs were performed in triplicate at 20°C.

In each case, the monolayer systems were compared to solutions containing the equivalent concentration of surfactant. This method represents a compromise, as even though the effects of the monolayer on the surfactant solution will be measured (as opposed to the effects of the monolayer and surfactant on water, had pure water been used for comparison), it is not known to what extent the monolayer will displace the surfactant from the surface. Indeed, one of the principle drawbacks associated with looking at interactions between systems at surfaces, especially when one is water soluble, is that it is difficult to assess how much of each component is present at the surface at a particular compression. This problem has been discussed in depth by a number of authors (e.g., Pethica, 1955; Alexander et al., 1986) and approaches to estimating the surface concentration have been outlined.

Fig. 1 shows the response of EPC on water and on a subphase of SLS at various concentrations below the CMC, showing the data over a comparable range of compressions. While plots are shown in terms of the area per phospholipid molecule, it should be appreciated that this area will represent the sum of the area occupied by the phospholipid molecule and any surrounding surfactant. The profile shows a gradual increase

in surface pressure as the film is compressed with a small (but reproducible) discontinuity seen at 27.3 Å^2/molecule which may represent a transition between different molecular packing states. Collapse of the film, indicated by the sudden change in slope of the profile, occurred at 22.1 Å^2/molecule. On addition of 0.0025% SLS, very little change was seen in the profile. However, on increasing the concentration to 0.005% w/v a marked shift in profile was observed which persisted up to concentrations of 0.025% w/v. The surface pressure increased in comparison to the EPC alone, as has been noted in previous studies on surfactant effects (e.g., Weingarten et al., 1991), while the collapse pressure also increased, although not in a clear rank order with regard to surfactant concentration. It is interesting to note that the discontinuity seen for the phospholipid alone is also seen when using SLS as a subphase, although this transition occurred at a greater molecular area in the presence of surfactant.

These results indicate that at low concentrations, the effects of addition of SLS on the phospholipid film appear to be small. However, on increasing the concentration, the surface pressure is higher at any compression due to the presence of both surfactant and phospholipid within the surface film. It is interesting to note that the increase in surface pressure is greater for surfactant solutions than for water. If the surface pressure was simply a function of the phospholipid alone, then one would expect the profiles to be largely similar to that obtained for water. Furthermore, if any extensive displacement of the surfactant by the EPC was taking place, one might expect to see a less marked increase in surface pressure, as the surface tension lowering (and hence surface pressure raising) effects of the surfactant would not be observed. The increases in surface pressure seen in Fig. 1 may indicate the presence of an interaction between the surfactant and the monolayer, involving the formation of a mixed film of EPC and SLS. As the film is compressed, the presence of this interaction may be hindering the SLS from simply dissolving into the subphase as the beams move together. Consequently, the film is effectively exerting a greater force on the beams in the presence of surfactant, which is reflected by the higher values of surface pressure.

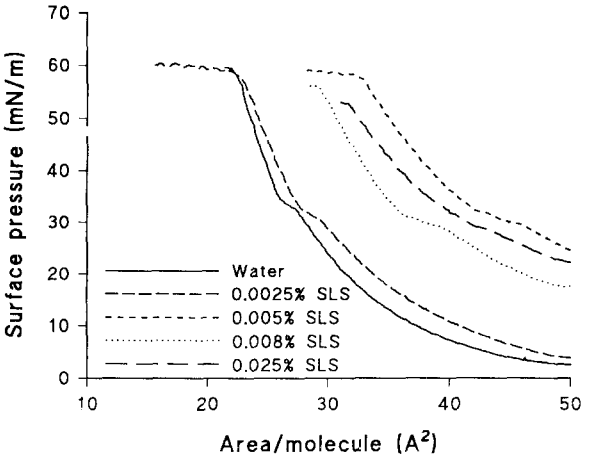

Fig. 1. π-area isotherms for egg phosphatidylcholine on solutions of sodium lauryl sulphate.

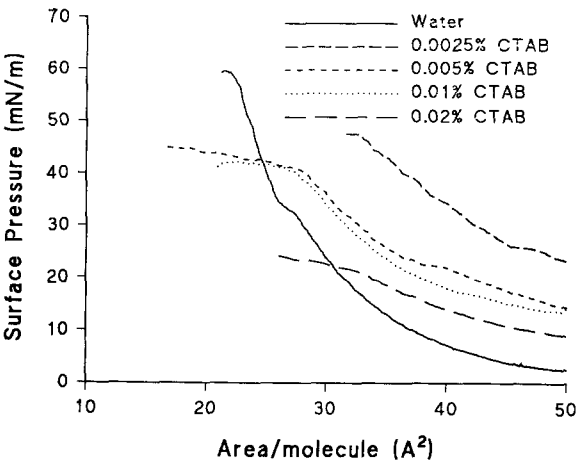

Fig. 2. π-area isotherms for egg phosphatidylcholine on solutions of cetyltrimethylammonium bromide.

When CTAB was used as the subphase (Fig. 2), a different concentration dependence was observed. The lowest concentration (0.0025% w/v) resulted in a marked increase in surface pressure, while on increasing the concentration the profiles showed a progressive decrease in surface pressure at any given compression. Furthermore, the

(negative) slopes of the profiles were considerably lower than for the EPC on water. This may be due to the CTAB disrupting the film formation process such that the phospholipid molecules are unable to pack together to form a coherent film, thus the surface pressure increases slowly over a much wider bath area. It is also noted that the discontinuity seen for EPC alone is not observed at higher surfactant concentrations, thus supporting the hypothesis that the CTAB is preventing film formation.

These results therefore indicate that SLS and CTAB interact with EPC monolayers via different mechanisms over the concentration range studied, with SLS showing evidence for an interaction with the film, resulting in a mixed film with a relatively high surface pressure. CTAB, however, provides less evidence for such an interaction under the conditions of study and may be acting by disrupting the film formation process. These differences may be partly due to the concentrations of the two surfactants used in this study, as while the ranges used were equivalent in both cases, the highest concentrations of CTAB used were approaching the CMC of this surfactant. Ruiz et al. (1988) have suggested that surfactant-induced release of liposomal contents (and by implication alterations to cell membrane permeability) takes place at concentrations below the surfactant CMC. The results presented here are compatible with this observation and suggest that it may be possible to use the technique as a means of predicting the membrane disruption effects of surfactants, or indeed drugs. While the limitations of the approach used here are appreciated and have been described above, these initial studies indicate that there is considerable potential for using this technique as a means of monitoring surfactant interactions with phospholipid films.

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