Eur Biophys J (2000) 29: 196–203

ARTICLE

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Formation of model lipid bilayers at the silica-water interface by co-adsorption with non-ionic dodecyl maltoside surfactant

Received: 6 September 1999 / Revised version: 25 February 2000 / Accepted: 25 February 2000

Abstract This present article describes a new and simple method for preparing model lipid bilayers. Stable and reproducible surface layers were produced at silica surfaces by co- adsorbing lipid with surfactant at the silica surface from mixed micellar solutions. The adsorption was followed in situ by use of ellipsometry. The mixed micellar solution consisted of a lipid (L-α-dioleoyllecithin) and a non-ionic sugar-based surfactant (n-dodecyl-β-maltoside). The latter showed, by itself, no affinity for the surface and could, therefore, easily be rinsed off the surface after the adsorption step. By first adsorbing from solutions with high lipid and surfactant concentrations and then, in succession, rinsing and re-adsorbing from solutions with lower lipid-surfactant concentrations, a dense-packed lipid bilayer was produced at the silica surface. The same result can be achieved in a one-step process where the rinsing, after adsorption from the concentrated solution, is performed very slowly. The thickness of the adsorbed lecithin bilayer after this treatment found was to be about 44 ± 3 Å, having a mean refractive index of 1.480 ± 0.004. The calculated surface excess of lipids on silica was about 4.2 mg m^{-2}, giving an average area per lipid molecule in the two layers of 62 ± 3 $Å^2$. The physical characteristic of the adsorbed bilayer is in good agreement with previously reported data on bulk and surface supported lipid bilayers. However, in contrast to previous investigations, we found no support for the presence of a thicker multi-molecular water layer located between the lipid layer and the solid substrate.

Key words L-α-Dioleoyllecithin · n-Dodecyl-β-maltoside · Adsorption · Lipid bilayer · Ellipsometry

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Introduction

Self-assembled phospholipid structures play an important role in biological processes. Examples include being substrates for serum protein adsorption in the initial stages of opsonisation (Malmsten 1998; Patel 1992; Semple and Chonn 1996) and being responsible for lipoprotein deposition at the endothelium surfaces of blood vessel walls in the early stages of atheroschlerosis (Greger and Windhorst 1996). Phospholipid surfaces are also of great importance to a number of biomedical applications, including intraveneous drug delivery, biomaterials, and biosensors. The development of simple and reproducible deposition methods for phospholipid layers on solid substrates is of great practical interest for such applications. Surface-supported lipid layers are, of course, also interesting as model surfaces in studies of adsorption of proteins and other biologically active molecules, and miscellaneous biophysical studies of membranes (Sackman 1996). Lipid bilayers, in particular, have a large potential in the design of biosensors, since they may be used as a medium for membrane-bound proteins or receptors.

The most commonly used methods for depositing phospholipid layers at solid supports include: the Langmuir-Blodgett deposition (Kop et al. 1984; Malmsten 1994; Swart 1990), spin coating (Malmsten 1995), and adsorption and subsequent collapse of phospholipid liposomes (Andree et al. 1993; Bayerl and Bloom 1990; Giesen et al. 1991; Johnson et al. 1991). All these methods have been proven useful, but they also suffer from different drawbacks. Langmuir-Blodgett (LB) deposition, for example, provides a means for deposition of lipid layers under controlled surface pressures. By this technique, an arbitrary number of layers may be deposited. The technique is, however, rather time consuming and sensitive to different system parameters as well as to surface defects. This makes the technique less interesting for large-scale biosensor applications, for instance, in vitro screening methods for candidate drugs. Moreover, LB layers are frequently quite unstable (Swart 1990). Spin coating, on the other hand, is a rather fast technique for the deposition of lipid layers at solid supports, at least above the lipid melting transition. The lipid layers obtained by this technique are, however, not so well defined. Spin coating is furthermore difficult to use in connection with deposition of membrane-bound proteins or receptor systems onto solid substrates. Spin coating is also very sensitive to the surface topography and cannot be used on, for example, polystyrene plates, which are typically used in solid phase diagnostics. Adsorption of liposomes is, finally, a quite attractive method, since membrane-bound proteins, and even functionally essentially intact receptor systems, may be transferred from cells to liposomes (Okumura et al. 1994; Suzuki et al. 1995). Although liposome adsorption has been used successfully for depositing phospholipid layers at solid surfaces, the success of this approach is strongly dependent on the pre-treatment of the lipid solution (Andree et al. 1993; Bayerl and Bloom 1990; Giesen et al. 1991; Johnson et al. 1991; Kop et al. 1984; Lindblom-Sethson et al. 1998; Winger and Chaikof 1998). The result of liposome adsorption is frequently controlled by surface nucleation phenomena and membrane fusion processes, which can impair on the reproducibility of the deposited layers.

The current study was carried out with the aim of elucidating if co-adsorption of lecithin with surfactant could be a useful alternative approach for depositing lipid bilayers on silica. A further goal was to study the kinetics of the layer build-up during adsorption and to measure and evaluate the end properties of the finally formed surface layer. The method used for this purpose was ellipsometry, which is a rapid non-destructive technique for in situ measurements of adsorbed layer properties as a function of time. The results obtained were finally compared with previously reported results on the properties of lipid bilayers adsorbed at surfaces as well as present in bulk solution.

Materials and methods

Materials

L-α-Dioleoyllecithin was purchased from Avanti Polar-Lipids. The molecular weight is 786 g/mol and the purity of the lecithin was >99% with respect to both its dioleyl chain length and the head group composition. The surfactant used to solubilize the lecithin was n-dodecyl-β-D-maltoside (purity >98%, molecular weight = 510.6 g/mol) purchased from Sigma Chemicals. The cmc of the surfactant is reported to be 1.67×10^{-4} mol/L (Hines et al. 1998) ($= 8.53 \times 10^{-5}$ g/L). In this paper, we refer to these substances as lecithin and DDM, respectively. Both substances were used as received, i.e. they were not subjected to any further purification steps.

Polished silicon test slides (p-type, boron-doped, resistivity 1–20 Ω cm) were purchased from Okmetic. The wafers were oxidized thermally in a saturated oxygen atmosphere at 920 °C for ≈1 h, followed by annealing and cooling in an argon flow. This procedure results in a SiO_2 layer thickness of ≈300 Å. The rms roughness of the substrates were about 1 Å, as measured by atomic force microscopy (results not shown). The oxidized wafers were then cut into slides with a width of 12.5 mm and cleaned according to the procedure described in Tiberg and Landgren (1993). The bare silica substrates were then treated in residual air in a plasma cleaner (Harrick Scientific Corporation, model PDC-3XG) for 5 min just before use. The surfaces were then rinsed off by a continuous flow of doubly distilled Millipore water, DD-MP. The silica surface were then, prior to the start of the adsorption measurements, allowed to stabilize in the solvent for at least 1 h.

Methods

Ellipsometry is a highly effective, rapid, non-destructive technique for time-resolved measurements of the thicknesses and refractive indices of adsorbed layers. In contrast to, for instance, neutron reflection, which under optimal conditions can provide detailed information on the segment density profile of the adsorbed layer, ellipsometry provides averaged values of the film properties. This drawback is compensated by the high sensitivity, its relatively simple handling, and the rapidness that enables kinetic adsorption studies (Tiberg 1996) to be carried out.

The technique relies on simple optical principles and is used to measure polarization changes occurring at oblique reflection of a polarized light beam from a surface (Azzam and Bashara 1989). These changes reflect the optical properties of the reflecting system through the relation

$$\tan\psi e^{j\Delta} = f(\lambda, \phi_0, \text{optical parameters}) \qquad (1)$$

between the optical response and the wavelength of the light, λ, the angle of incidence, ϕ_0, and the optical properties of the reflecting system. The measured quantities ψ and Δ, often referred to as ellipsometric angles, reflect the difference in the polarization state between the incident and the reflected light. In the case of a reflection at one interface, the optical parameters of relevance are the refractive indices of the two phases. When planar, isotropic films are located between two bulk media, the overall reflection coefficients also depend on the thickness and refractive indices of each of these layers.

The instrument used in this study was an automated Rudolph Research thin-film ellipsometer, type 43603-200E, equipped with high-precision step motors from Berger-Lahr, type VRDM 566, and controlled by a personal computer. The experimental set-up as well as a description of the procedure for in situ characterizations of thin films adsorbed on layered substrates are given in Tiberg and Landgren (1993).

The systems discussed in this work were always treated as composed of a number of homogenous and optically isotropic layers located between the substrate and the surrounding solution. All interfaces between these layers were assumed to be perfectly flat. The bulk silicon has a complex refractive index, $N_2 = n_2 - jk_2$, whereas both the silicon oxide and the adsorbed film are transparent (i.e. $k_i \approx 0$) and, thus, are characterized by thicknesses, d_1, and d, and the real parts of the refractive indices, n_1 and n, respectively. A maximum of two of these parameters can be determined from a single set of ψ and Δ readings, provided that the rest of the parameters in Eq. (1) are known. All the measurements presented in this work were performed at $\lambda = 4015\,\text{Å}$ and $\phi_0 = 67\,°$.

In order to make accurate measurements of the adsorbed layer properties, knowledge of the substrate properties is required. Since four unknown parameters (n_2, k_2, n_1 and d_1) must be determined, at least four measured parameters are needed. For this purpose, a new methodology was developed, where two sets of ψ and Δ were determined in different ambient media (air followed by water). This substrate characterization procedure is described in Landgren and Jönsson (1993) and Tiberg and Landgren (1993). The values of n_2, k_2, n_1 and d_1 determined at $\lambda = 4015\,\text{Å}$ were: $n_2 = 5.505 \pm 0.005$, $k_2 = -0.37 \pm 0.03$, $n_1 = 1.480 \pm 0.03$ and $d_1 \approx 300\,\text{Å}$ (varying somewhat between different substrate batches).

When the optical properties of the substrate had been determined, a controlled amount of adsorbate was added to a temperature-controlled cuvette (25.0 ± 0.1 °C), and the ellipsometric angles ψ and Δ were then monitored as a function of time. Stirring was performed with a magnetic stirrer at about 300 rpm and rinsing by a continuous flow (20 mL/min) of DD-MP water through the cuvette containing 5 mL liquid. The maximum time-resolution of our null ellipsometer is 1–2 s; however, to obtain high-precision measurements, we allowed approximately 3–4 s for measuring each set of ψ and Δ. Knowing the substrate properties, the average refractive index, n, and the mean thickness, d, of the adsorbed layer were calculated numerically from the ψ and Δ readings by a version of Eq. (1) derived for the optical four-layer model used to describe the system under study. Both n and d were, as mentioned calculated, based on the assumption of adsorbed layer uniformity. The layer thickness measured by ellipsometry has previously been found to agree, within a few ångströms, with the corresponding thickness measured by, for example, neutron and X-ray scattering, neutron reflectivity, streaming potential, and surface force measurements (Böhmer et al. 1992; Gellan and Rochester 1985; Lee et al. 1989; McDermott et al. 1992; Rutland and Senden 1993; Tiberg 1996; Tiberg and Ederth, in preparation).

The calculated values of n and d were also used to calculate the amount adsorbed or surface excess, Γ, according to the formula

$$\Gamma = \frac{(n - n_0)}{\mathrm{d}n/\mathrm{d}c} d \tag{2}$$

where n_0 is the refractive index of the bulk solution and $\mathrm{d}n/\mathrm{d}c$ is the refractive index increment (=0.154 for dioleoyllecithin).

The effects of errors in the measured ellipsometric angles ψ and Δ on the calculated values of n and d were estimated earlier. For typical experimental conditions, the errors in ψ and Δ are normally distributed with standard deviation 0.001 ° and 0.002 °, respectively. At small surface coverages, $\Gamma < 0.5\,\mathrm{mg\,m^{-2}}$, the relative error in the thickness is rather high (see Tiberg 1996). For large adsorbed amounts ($\Gamma > 2\,\mathrm{mg\,m^{-2}}$), the errors rapidly decrease to values below 5%. The same trend was observed for the error in the refractive index. The errors in the adsorbed amount are much smaller. The maximum error in Γ in the low coverage limit ($\approx 0.1\,\mathrm{mg\,m^{-2}}$) is about 15% and decreases to less than 1% for $\Gamma > 2\,\mathrm{mg\,m^{-2}}$. This is due to the fact that the errors in d and n are covariant in such a way that they to a large degree cancel out when Γ is calculated. Besides these errors, there are also systematic errors. The effects of these have been discussed previously. Their magnitude was generally much smaller than the errors due to ψ and Δ fluctuations.

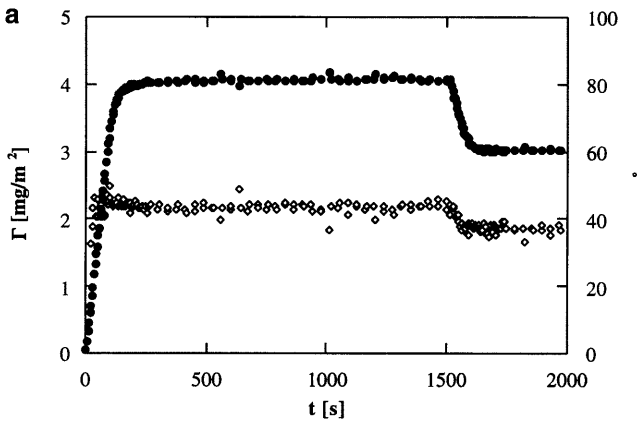
Results and discussion

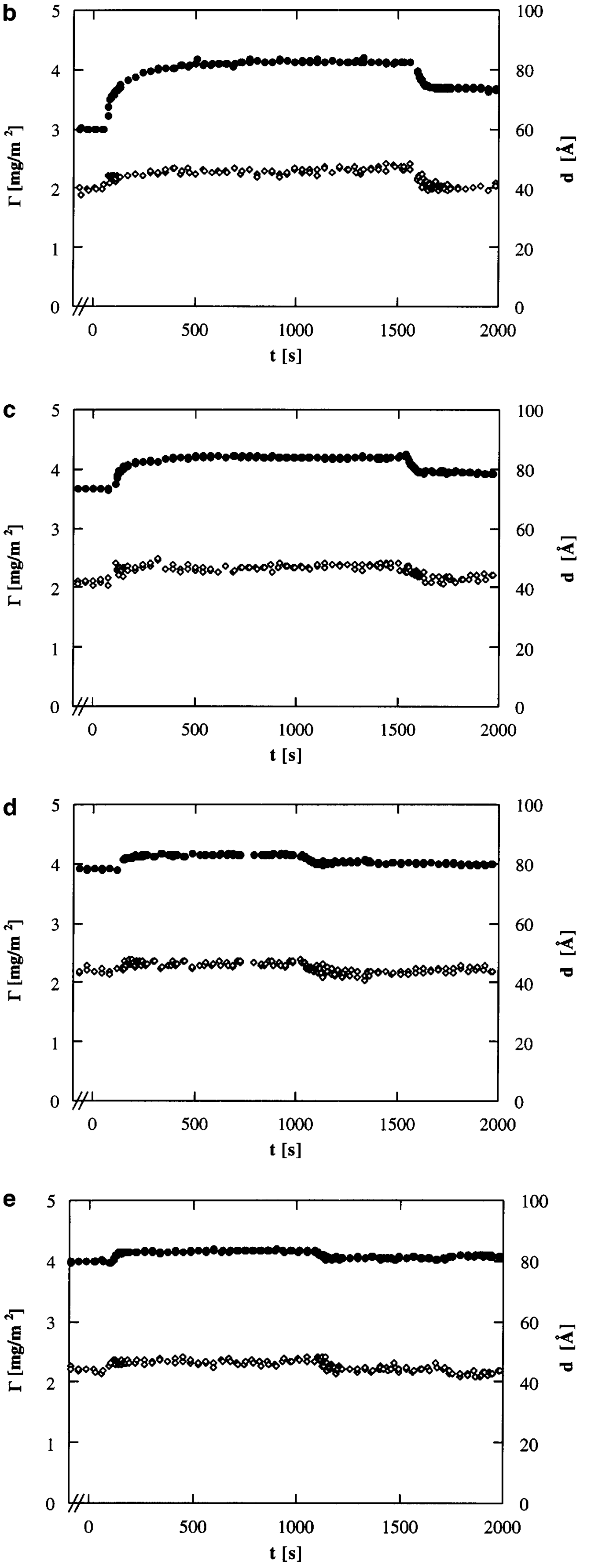
The work presented in this article shows a new strategy for preparing model lipid surfaces by adsorbing mixed micelles of, in this case, L-α-dioleoyllecithin (referred to below simply as lecithin) and n-dodecyl-β-D-maltoside surfactants (called DDM) on silica. The approach used was to start adsorbing from a relatively high concentration solution of lecithin and DDM and then in succession rinse and re-adsorb from solutions of lower concentration. The ratio of lecithin:DDM that was used was 1:6 throughout. It is noteworthy that the lecithin-DDM solutions become turbid at higher lecithin fractions, probably indicating the presence of vesicles. The reason for choosing DDM as the solubilizing agent for lecithin was twofold. First, the DDM surfactant does not by itself have an affinity for the silica surface (i.e. it was not found to adsorb on silica), which should result in a preferential adsorption of lecithin at this interface. Second, DDM is highly water soluble and still efficient as a lipid solubilizing agent, unlike the shorter-chain sugar-based surfactants.

It has been shown in previous work that surfactants with low solubility (e.g. as determined by the cmc) become more preferentially adsorbed at hydrophilic surfaces as one lowers the total surfactant concentration and approaches the cmc of the low solubility compound (Brinck and Tiberg 1996; Brinck et al. 1998; Eskilsson and Tiberg 1998). This was, for instance, observed for pairs of poly(ethylene glycol) monoalkyl ethers with identical hexa(ethylene glycol) head groups and different alkyl chain lengths. These surfactants adsorb as aggregates at silica with similar characteristics as their corresponding bulk structures (Tiberg et al. 1994b). Indeed, the surface aggregate composition was found to be similar to the bulk composition. With decreasing concentration, both bulk and surface aggregates become dominated by the low solubility compound, a phenomenon that is predicted quite simply by ideal solution theory. By combining the solubility effect on adsorption with a preferentially interacting head group, it should be possible to produce adsorbed layers from micellar systems that are almost completely dominated by the low solubility compound. A further interesting fact learned in the previous work is that the desorption rate from hydrophilic (silica) surfaces covered by surfactants is proportional to the solubility of the surfactant (Tiberg et al. 1994a). Molecules with very low solubility, like lecithin, are hence "irreversibly" absorbed with respect to rinsing with water. With the above in mind, we hoped to be able to produce well-defined, dense, and irreversibly bound lipid bilayers. We chose to work with a lecithin-DDM mixture, which fulfils the success criteria mentioned above.

Figure 1a–e shows the time evolution of the thickness and surface excess of adsorbed lecithin-DDM layers at silica. The different figures show the evolution after successive addition and rinsing cycles, starting from high lecithin-DDM concentrations and a bare silica surface

Fig. 1 a Time evolution of the adsorbed layer thickness (*open diamonds*) and the surface excess (*filled circles*) during adsorption onto bare silica from a 1.14×10^{-4} mg/L micellar solution of lecithin and DDM (1:6 by weight), and then after 1500 s, during rinsing with a continuous flow (20 mL/min) of DD-MP water. **b–e** The corresponding evolution during successive re-adsorption from lecithin-DDM solutions (1:6 by weight) of concentrations 2.28×10^{-5} g/L, 1.14×10^{-5} g/L, 5.7×10^{-6} g/L, and 2.85×10^{-6} g/L, respectively. The time scale is between each measurement adjusted so that 0 s coincides approximately with the moment of injection of a new lecithin-DDM stock solution into the cuvette





and going to lower concentrations and an increasingly dense adsorbed layer dominated by lecithin. Figure 1a shows a relatively rapid linear increase of the surface excess with time after the first addition of the lecithin-DDM solution into the cuvette. The total bulk concentration of adsorbent in the cuvette was 1.14×10^{-4} g/L. The ratio of lecithin:DDM was 1:6 in all adsorption runs. A plateau adsorption value of about 4.1 mg/m^2 is in this first adsorption cycle reached within about 250 s. A plateau layer thickness is established much earlier, which is consistent with the fact that the molecules form aggregates at the surface with a well-defined thickness ($\approx$45 Å). Further adsorption only results in an increased size (or density) of these surface aggregates. When rinsing with water ($\approx$20 mL/min) is started at around 1500 s, we note that the surface excess first decreases rapidly and then a new plateau adsorption value is established around 3.0 mg/m^2, i.e. rinsing results in desorption of about 27% of the original surface excess. Since the molecular solubility of DDM is orders of magnitude higher than lecithin and the desorption rate is proportional to solubility, it is quite safe to state that the desorbed fraction is almost exclusively DDM. Note also that DDM has no affinity for the surface by itself. Adsorption at the silica-water interface could be not detected for this surfactant. It is not surprising that we found the desorbed fraction to be lower than the corresponding bulk fraction of DDM. This is in agreement both with the higher solubility of DDM compared to lecithin and the insignificant DDM-surface interactions with silica. We conclude that substantial desorption of mainly DDM is observed when rapidly diluting the solution from a relatively high lecithin-DDM solution with pure water, but this is still a much smaller fraction than the bulk ratio of DDM in solution. The fact that desorption is substantially larger than the thickness decrease indicates that the surface coverage of the silica by the lecithin-DDM bilayer (or bilayer segments) decreased during rinsing.

However, the problem of desorption and incomplete layer formation can be reduced by lowering the lecithin-DDM concentration during adsorption (see Fig. 1b–e). The graphs show re-adsorption onto the lecithin-covered silica surfaces from the previous adsorption-rising cycle. The re-adsorption was carried out from lecithin-DDM solutions (1:6 by weight) with successively decreasing bulk concentrations. In the measurement depicted in Fig. 1b, the lecithin-DDM concentration was lowered by a factor 5 from the previous measurement in Fig. 1a. We see clearly that the re-adsorption process (started at about 0 s) results in a plateau surface excess of 4.2 mg/m^2 and a thickness of about 45 Å, i.e., similar to the levels reached in the first adsorption measurement. However, a much smaller fraction is desorbed from the surface after rinsing with DD-MP water. This is consistent with the notion that the surface bilayer (bilayer segments) is increasingly populated by the less soluble lecithin molecules the lower the concentration is during adsorption. Note that lecithin due to its low molecular solubility in water can be considered irreversibly adsorbed on the time-scale of our measurements. In Fig. 1c to Fig. 1e, the same re-adsorption rinsing procedure was used as mentioned above. The only difference is that the lecithin-DDM concentrations during re-adsorption were lower (see figure caption). The result of this concentration decrease is that the desorbed fraction during rinsing (after re-adsorption) becomes smaller. In the last two runs, the surface excess and thickness only change by a few percent during rinsing. We infer that the end result is a well-defined lecithin bilayer. The bilayer picture is consistent with the adsorbed layer thickness of 44 ± 3 Å, which is substantially thicker than the maximum extension of one lecithin molecule (estimated to be around 25 Å). Furthermore, the layer thickness agrees well with the bilayer thickness measured in the lamellar phase of dioleyllecithin by Parsegian and co-workers (Lis et al. 1982), who found the bilayer thickness to be between about 43 Å and 35 Å, depending on the water content. The thickness measured at lower water content is in perfect agreement with the ellipsometric layer thickness of the surface-immobilized bilayer obtained in the current work. An identical bilayer thickness of 43 Å was also measured by neutron reflectivity for lipid (asolectin) bilayers on silica by Krueger et al. (1995). These bilayer structures were formed by fusion of lipid vesicles onto a LB-deposited lipid monolayer. Using the same technique, Johnson et al. (1991) also measured a thickness of 43 Å for dimyritoylphosphatidylcholine (DMPC) bilayers adsorbed at silica from a vesicular solution. Note, however, that it was inferred in this study that a 30 Å water layer was present between the DMPC bilayer and the surface. A similarly high value of 17 Å was deduced in a deuterium nuclear magnetic resonance study (Bayerl and Bloom 1990). Our ellipsometric data do not seem compatible with the existence of such a thick water layer between the adsorbed bilayer and the solid surface. Introducing 30 Å water layer results in lipid layer thickness of 17 Å and a refractive index of 1.9, which clearly are unreasonable values. The corresponding numbers for a 17 Å water layer are 23 Å and 1.65, respectively. Indeed, with regards to both the expected lipid bilayer thickness and the refractive index, it seems quite unlikely that any layer of water between the bilayer and the surfaces is thicker than 5 Å, which is compatible with the results of Krueger et al. (1995). Note that in the neutron reflectivity study of Johnson et al. (1991) the reflectivity curves were reported to be fitted well also without introducing the 30 Å water layer. This option was, however, rejected, based on two grounds: the fact that the lipids have been found to diffuse laterally on quartz [earlier reported by Bayerl and Bloom (1990)] and that the zwitterionic head interacts favourably with water. Both arguments are questionable and represent no conclusive evidence for the presence of a thick water layer between the adsorbed layer and the surface. Furthermore, if such a water layer would be present and if the head group interacts so strongly with water, then it seems rather difficult to ascribe a driving force for bilayer adsorption at silica. We conclude this discussion by stating that the measured layer thickness for the co-adsorbed dioleoyllecithin layers agrees well with the results obtained in earlier studies for similar lipids systems. However, the presence of a multi-molecular water layer between the silica surface and the bilayer is not supported by our experimental results.

In Fig. 2a and b, the refractive index evolution is shown together with the thickness for the same experiments as are shown in Fig 1a and e, respectively. It is in these graphs worth noting that, after rinsing, the refractive index is higher in Fig. 2b. than in Fig. 2a. This agrees well with our conclusion that the bilayer is less dense or possibly fragmented after an adsorption (high concentration) rinsing cycle. The re-adsorption (low concentration) rinsing cycle results in a more dense structure, agreeing with the picture of a dense lecithin bilayer having been formed. The average area per lecithin molecule in the two layers forming the bilayer is $62 \pm 3\,\text{Å}^2$, as calculated from the surface excess data following rinsing. Again, this figure can be compared to the area per molecule given in the study by Parsegian and co-workers (Lis et al. 1982). Depending on the water content in the lamellar phase, areas between $60\,\text{Å}^2$ and $80\,\text{Å}^2$ are reported in this work. For an area of $62\,\text{Å}^2$, the corresponding layer bilayer thickness was found to be just above 40 Å, which again is in excellent agreement with our results. Therefore, we may conclude that adsorption results in the formation of a lecithin bilayer structure with comparable properties to the bilayer structures observed in bulk solution.

Not entirely evident as yet is the issue of why we, in the procedure of preparing the bilayer, started from a high concentration and subsequently performed successive rinsing re-adsorption cycles to build up the lecithin layer instead of directly building up the bilayer from a low surfactant concentration. The reason for this is that the adsorption process is very slow at lower concentrations. Figure 3 shows the time evolution of the surface excess and layer thickness when adsorbing from a 1.14×10^{-5} g/L solution of lecithin-DDM (1:6 by weight). It is clearly seen that the absorbed layer build-up takes a very long time at this concentration, which still is quite high in comparison to that used in Fig. 1e. Indeed a surface excess value of only about 1.5 mg/m^2 is reached after 8000 s. The thickness is, however, larger than 40 Å during the entire adsorption measurement, indicating that the bilayer structure is formed very early in the adsorption process. The most likely evolution is that the size and/or number of bilayer segments increases throughout the adsorption process. The reason for the slow kinetics during direct adsorption is unclear. One explanation may be that the bulk aggregates at this concentration are larger and thus diffuse slower or that some energy barrier exists for the adsorption of these aggregates at intermediate coverage. However, the changes in the bulk composition during the adsorption of lecithin seem to be the most plausible explanation. This would lead to an increasing fraction of DDM with low surface affinity in the bulk aggregates and hence a

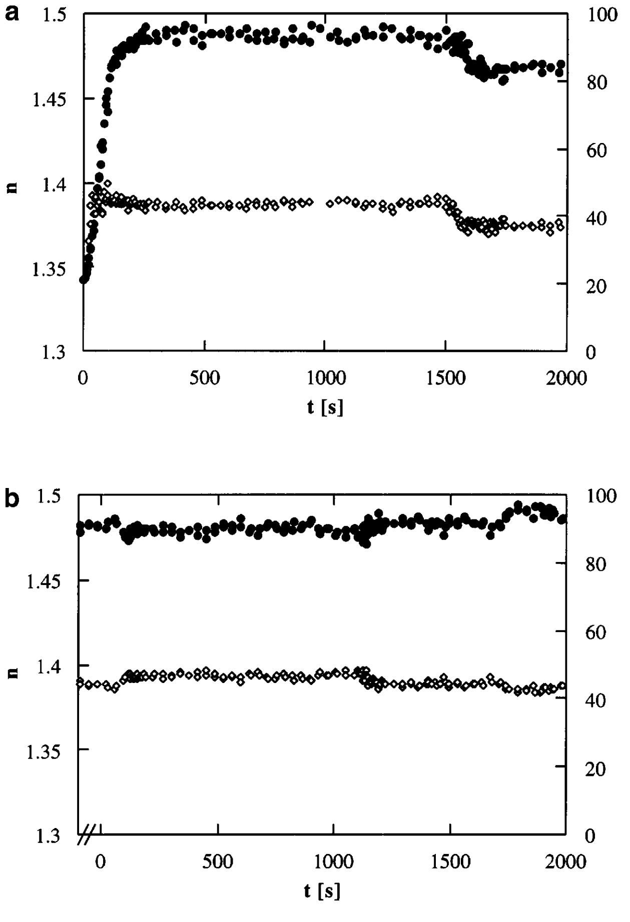


Fig. 2a, b Time evolution of the refractive index (*filled circles*) and thickness (*open diamonds*) for the experiments shown in Fig. 1a and e, respectively

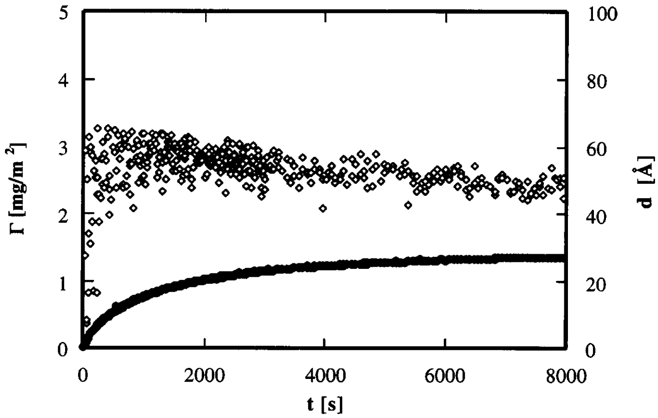


Fig. 3 Time evolution of the adsorbed layer thickness (*open diamonds*) and surface excess (*filled circles*) when adsorbing onto bare silica from a 1.14×10^{-5} g/L solution of lecithin and DDM (1:6 by weight). Note that the adsorption kinetics observed at this concentration is very slow

reduced tendency for adsorption. Further studies are needed to clarify this tissue.

As is understood from the discussion above, an adsorption and dilution scheme is necessary for producing dense model lipid surfaces by adsorption from mixed micellar solutions. However, it is important to stress that this must not be performed by repeated (and time consuming) re-adsorption dilution cycles as described above. A similar end-result was indeed achieved by adsorbing from a high concentration and then slowly diluting (rinsing) the solution with a continuous flow of 0.5 mL/min DD-MP per minute (results not shown). The shift of the local equilibrium conditions just outside the adsorbed layer during dilution results in back-diffusion of the less soluble lecithin towards the surface. The slow dilution rate ensures that the local equilibrium can be kept outside the adsorbed layer. The same phenomenon has previously been observed experimentally for pairs of nonionic surfactant, having different solubilities (Brinck and Tiberg 1996; Brinck et al. 1998). In the same study, a theoretical model was presented which successfully was used to predict adsorption kinetics and diffusion behaviour for two-component systems.

Finally, it is worth mentioning that adsorption was also studied from turbid (most likely vesicular) solutions. We were not able under these conditions to produce well-defined bilayer structures as obtained during adsorption from the non-turbid micellar phase. Besides not giving the same end-properties, the adsorption results turned out to be quite irreproducible for the presently investigated system.

Conclusions

We have shown that it is possible by co-adsorption of dioleoyllecithin and dodecyl DDM surfactants followed by rinsing to produce bilayers composed more or less exclusively of lecithin. By re-adsorbing successively from decreasing total bulk concentrations, we managed to prepare a dense bilayer structure with characteristics similar to those observed in bulk solution. The adsorbed bilayer thickness, refractive index and mean area per molecule were found to be 43 ± 3 Å, 1.480 ± 0.004, and 62 ± 3 Å^2, respectively. It is believed that this method for producing a stratified lipid bilayer can be used in diverse applications, e.g. in studies of interaction forces and protein adsorption, high throughput screening of candidate drugs, biosensors, and solid phase diagnostics and synthesis. The method presented is not limited to dioleoyllecithin, but can be used for any lipid with low solubility and affinity for silica. The technique can most likely also be used for deposition lipid bilayers on hydrophilic surfaces other than silica, for instance, mica and metal oxides. Monolayer deposition on hydrophobic surfaces should also be possible, although the surface affinity of the lipid and the surfactant in this case is more similar.

Acknowledgements This work was supported by the Swedish Foundation for Strategic Research (SSF) and the Swedish Research Council for Engineering Sciences (TFR). M.M. also acknowledges financial support from the EU-FAIR project CT96-1216.

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