

Synthetic Bilayer Wetting on SiO₂ Surfaces

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Received: October 10, 1997; In Final Form: January 22, 1998

Wetting on SiO₂/Si wafers in the presence of dioctadecyldimethylammonium bromide (DODAB) bilayer vesicles is determined as a function of DODAB concentration and time of interaction between surface and vesicles. In 10 mM TRIS buffer at pH 7.4, the dynamic contact angle (θ) displays a maximum at an intermediate DODAB concentration (C) that corresponds to a minimum of contact angle hysteresis and is consistent with optimization of bilayer deposition. In pure water, θ is smaller than the contact angle for the bare surface consistently with adhesion of collapsed vesicles that carry water. In buffer, angles are larger than those for the bare surface, suggesting bilayer deposition under water and its conversion to a monolayer as the bilayer-bearing surface recedes into air. At a given C , θ against the interaction time (t) has an inverted-bell shape, indicating first vesicle adhesion, then bilayer deposition (at intermediate times), and finally further vesicle adhesion onto the deposited bilayer. Two major wettability parameters characterize bilayer deposition on the solid surface: minimization of contact angle hysteresis and maximization of the measured contact angle. Determination of dynamic contact angles is proposed as a quick and efficient technique to distinguish between vesicle adhesion and bilayer deposition on solid surfaces in general.

Introduction

The interaction of water-soluble surfactants with solid polymeric or mineral surfaces has been intensively and extensively studied.^{1–3} However, the potential of bilayer-forming amphiphiles as interface agents able to modify solid surfaces remains hitherto poorly explored. Bilayer-forming amphiphiles assemble on solid particles or planar surfaces either as a bilayer, a monolayer, or adhered vesicles depending on the nature of the amphiphile and surface and on interactions driving amphiphile deposition.^{4–16} Bilayer deposition of dioctadecyldimethylammonium bromide (DODAB) and sodium dihexadecyl phosphate (DHP) from vesicles onto oppositely charged polystyrene microspheres was reported.⁴ There is an electrostatically driven vesicle adhesion to the latex that is followed by bilayer deposition.⁵ Thereafter, as amphiphile concentration increases, vesicles adhere to the bilayer-covered latex with or without disruption, depending on nature of the synthetic amphiphile.⁵ In general, the interaction between bilayer vesicles and solid surfaces in the form of particulates^{6–15} or planar surfaces^{16,17} still lacks characterization from the point of view of exact physical parameters. Recently, the physical adsorption of bilayer-forming amphiphiles on hydrophilic silica, in particular, was shown to depend on previous centrifugation of the vesicle sample, pH, buffer, temperature, and physical state of the bilayer.¹⁴ In fact, establishing suitable experimental conditions for the occurrence of bilayer deposition as well as an efficient and quick methodology to ascertain whether bilayer deposition indeed took place on a solid surface is still a problem for those interested in producing supported bilayers. Many applications of supported bilayers in biotechnology are presently hampered by the poor reproducibility of various bilayer deposition

methods. The process of bilayer deposition on solid substrates is indeed thought as “magic”. Therefore, its description in terms of exact physical parameters such as the contact angle¹⁰ or surface roughness¹⁶ may significantly improve our understanding of the underlying physicochemical processes on a molecular scale.

In this work, we propose the determination of dynamic contact angles as a major tool to distinguish between vesicle adhesion and bilayer deposition on a solid surface. Minimization of contact angle hysteresis and maximization of the contact angle value are established as precise criteria to identify bilayer deposition on solid surfaces in general. In addition, the influence of time and DODAB concentration on formation of bilayers or adhered vesicles on planar SiO₂ surfaces is described.

Material and Methods

Dioctadecyldimethylammonium bromide, DODAB (99.9% pure), was obtained from Sigma Chemical Co. (St. Louis, MO). Analytical determination of DODAB concentration was performed as previously described.^{4,5} All other reagents were analytical grade and used without further purification. Water was Milli-Q quality.

Small DODAB vesicles were prepared by sonication with the tip.^{4,18} Thereafter, the dispersion was centrifuged (14 000 rpm/h) to eliminate multilamellar vesicles and titanium ejected from the tip. A typical vesicle mean diameter is 86 nm.⁴

Silicon monocrystalline slides (<100> for crystal orientation, P type, 10–20 Ω cm of resistivity) of 0.4 mm mean thickness were cut with a diamond tip in order to obtain rectangular planar surfaces (25 \times 15 mm). Before thermic oxidation, cleaning of the silicon surface was performed in accordance with standard procedures for silicon uses in microelectronics.¹⁹ Rapid thermic oxidation²⁰ was carried out under dried oxygen (less than 5 ppm water) at 1150 $^{\circ}$ C/500 s using a Heatpulse 410T (A G Associates). A thickness of about 30 nm for the SiO₂ layer

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was determined using a Rudolph ellipsometer (Autoel IV Nir-3/4D/SSI). Two SiO₂/Si/SiO₂ slides of the same dimensions were glued at their rough faces with the smooth silicon oxide surface to the outside. Routinely, this slide was cleaned with deionized water/ethanol/deionized water/isopropyl alcohol/deionized water in this sequence. Thereafter, the smooth SiO₂ surfaces were considered clean with respect to occurrence of deposited organic films or particles and attached to the dynamometer balance.

Dynamic contact angles were determined using the extended Wilhelmy plate or wetting-balance method.^{21–23} Force–depth profiles for the slide under immersion or emersion in water or in DODA dispersions were obtained using a dynamometer (Byk-Labotron, Germany) as previously described.¹⁰ Contact angles θ were calculated from eq 1:²²

$$\cos \theta = (1/p\gamma)(F + \rho gAh) \quad (1)$$

where p = perimeter of slide (cm), γ = surface tension (dyn/cm), F = force on slide (dyn), ρ = density of water (g/mL), g = 980.2 (dyn/g), A = slide cross-sectional area, and h = depth of immersion (cm).

The equilibrium surface tension at 25 °C was measured as a function of DODAB concentration from 0 up to 2 mM DODAB using a Du Nouy ring, and the dynamometer itself was set to the surface tension mode. For surface tension measurements, 20 mL of DODAB dispersion was added to a polypropylene beaker (4 cm diameter) so that equilibration of the amphiphile with the air/water interface was always over the same total air/water interfacial area of 12.56 cm². Surface tension was measured as a function of DODAB concentration either in pure water or in 10 mM TRIS buffer at pH 7.4 or as a function of time after adding the DODAB dispersion to the beaker.

Results and Discussion

Effect of Equilibration Time and DODAB Concentration on the Surface Tension at the Air–Water Interface. Typical DODAB dispersions obtained by sonication in water display equilibrium surface tensions close to that of pure water up to 2.0 mM DODAB (Figure 1A). This is shown at three different equilibration times for vesicles equilibration with the air/water interface (Figure 1A). In pure water, at room temperature, surface tension is not affected by the equilibration time as shown at three different DODAB concentrations (Figure 1B). In 10 mM TRIS buffer at pH 7.4, the surface tension decreases as a function of DODAB concentration at three different equilibration times (Figure 1C) and as a function of equilibration time of the vesicle dispersion with the air/water interface at three different DODAB concentrations (Figure 1D). At high DODAB concentrations, large equilibration times, and 10 mM TRIS, surface tension becomes significantly smaller than the surface tension of water, which is 72 mN/m, though the smallest surface tension attained is still relatively high: ca. 47 mN/m, at 1.7 mM DODAB in 10 mM buffer after 3 h equilibration time (Figure 1C,D). Formation of a DODAB monolayer at the air–water interface from sonicated vesicles in dispersion is expected to decrease the surface tension. Thus, relatively high DODAB concentrations, rather long equilibration times (2–3 h) and a certain ionic strength (as given by the 10 mM TRIS buffer), are required for DODAB adsorption from sonicated vesicles at the air–water interface. This result confirms that these vesicle-forming amphiphiles are highly insoluble in water, though at 10 mM monovalent salt (in 10 mM TRIS buffer) DODAB solubility seems to increase, possibly due to salt-induced hydrophobic effects in the bilayer that would direct the vesicles

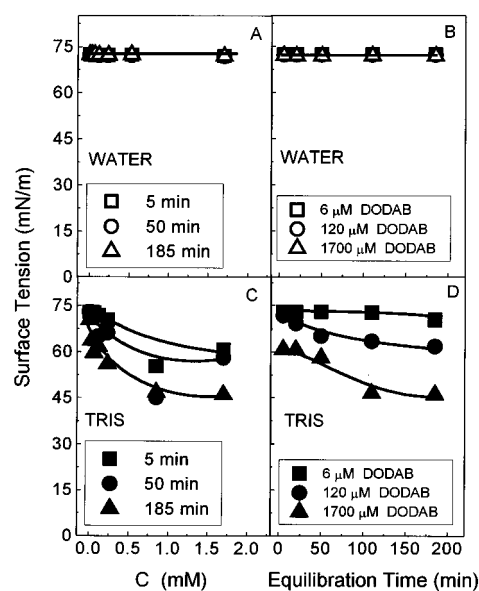


Figure 1. Effect of DODAB concentration (C) on the surface tension γ (mN/m) at the air–water interface for three different equilibration times of the dispersion in the beaker (A, C). Effect of the equilibration time of the dispersion in the beaker on the surface tension for three different DODAB concentrations (B, D). DODAB vesicles were prepared in water (A, B) or in 10 mM TRIS buffer at pH 7.4 (C, D).

to the air–water interface so that a DODAB monolayer would start to form. This agrees with the observation in the literature that a monovalent salt such as NaCl can induce fusion of DODAB vesicles.^{25,26} For dihexadecyldimethylammonium acetate (DHDA), surface tension steeply decreases from 72 to 30 mJ/m² at about 0.10 mM surfactant after 1 h equilibration time.²⁶ The C18 double-chained amphiphile in this study is less soluble than DHDA. DODAB molecules are less free to diffuse from vesicles to the air–water interface so that, in pure water, surface tension remains equal to the surface tension of water, and in 10 mM salt, a significant decrease of surface tension only occurs at large equilibration times and DODAB concentrations.

Optimization of DODAB Bilayer Deposition from Dynamic Contact Angles. In Figure 2, the dependence of apparent mass of the wafer, in milligrams, as a function of immersed depth (h) which is directly related to time (t) is displayed for the advancing (immersion) (Figure 2A) and receding (emersion) (Figure 2R) surface into or from a 33 μM DODAB dispersion of sonicated vesicles in pure water. There is a linear relationship between apparent mass and immersed depth as expected from eq 1. Extrapolation of the straight line to $t = 0$ ($h = 0$) yields the intercept which is directly used to obtain the contact angle.

In water, all contact angles determined over a range of DODAB concentrations are significantly smaller than the angle obtained for the bare SiO₂ surface, indicating an increased surface hydrophilicity in the presence of DODAB vesicles (Figure 3a). For the bare SiO₂ surface in water, advancing and receding angles are 87° and 68°, in close agreement with previous data from the literature.²⁸ In 10 mM TRIS at pH 7.4, the contact angle displays a maximum as a function of DODAB concentration for ca. 0.5 mM DODAB (Figure 3b). This maximum was not observed in water (Figure 3a). Furthermore, angles in TRIS become significantly larger than those obtained in pure water over an intermediary range of DODAB concentrations (Figure 3a); i.e., there is a maximum in surface hydrophobicity at 0.5 mM DODAB. Most interesting is the obser-

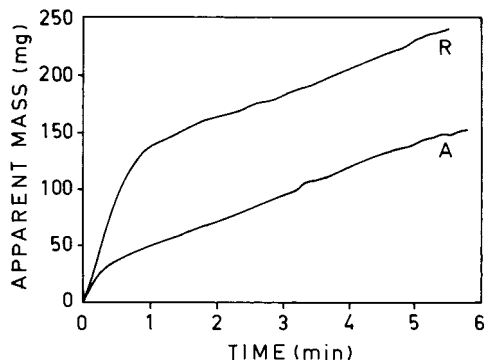


Figure 2. Apparent mass of slide, in milligrams, as a function of time, in minutes, for advancing, A, or receding, R, procedures applied to the SiO₂ surface. One should recall that advancing or receding rates are constant and equal to 1.5 mm/min so that time measured directly reflects depth of immersion for the SiO₂ slide. The slide is under immersion (A) or emersion (R) into or from, respectively, a 0.033 mM DODAB dispersion of small vesicles prepared in pure water. Surface tension and apparent mass were independently measured at 5 min of equilibration time for the DODAB dispersion in the vessel. The values obtained—72 mM/m for the surface tension and 25.9 (A) or 118.7 mg (R) for apparent mass at time zero (extrapolated from the linear portion of the curve)—were used for calculation of the dynamic contact angles (83.7° and 59.5°, respectively).

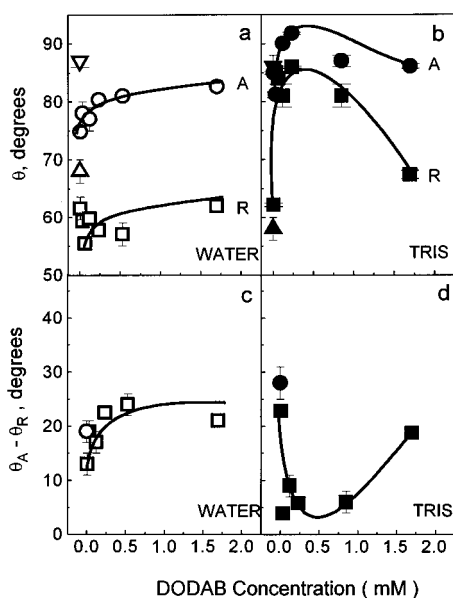


Figure 3. Effect of DODAB concentration and intervening medium on advancing (θ_A) or receding (θ_R) dynamic contact angle for SiO₂ surfaces (a, b) and on contact angle hysteresis (c, d). In (a) and (c), the medium is pure water. In (b) and (d), the medium is 10 mM TRIS buffer, pH 7.4. The single different symbol in each subfigure represents the contact angle measured for the bare SiO₂ surface, in the absence of DODAB vesicles. Measurements were taken at 2 h interaction time.

vation that contact angle hysteresis as a function of DODAB concentration is minimized and practically equal to zero at 0.5 mM DODAB (Figure 3d). Figure 5 presents a possible interpretation for these results. In water, vesicle adhesion to the surface would occur so that receding this assembly would generate collapsed vesicles that would still carry water, thereby causing an apparent increase in surface wettability (Figure 5A). In TRIS, vesicle adhesion followed by vesicle rupture and bilayer deposition under water would generate a hydrophobic amphiphile monolayer upon bringing the bilayer-covered surface into contact with air (Figure 5B). Reinforcing this model, DODAB adsorption isotherms onto hydrophilic silica particles in water or in 10 mM TRIS, at pH 7.4, demonstrated bilayer

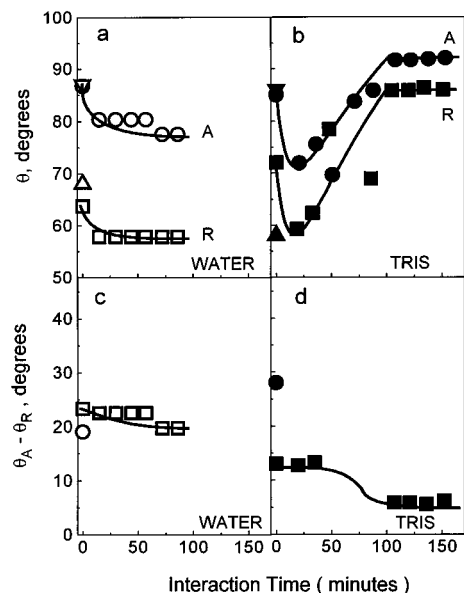


Figure 4. Effect of interaction time between SiO₂ surface and DODAB vesicles at 0.235 mM DODAB on dynamic contact angles (a, b) and on contact angle hysteresis (c, d). Angles were successively determined as a function of time, taking the start of the first immersion of the slide as time zero. In (a) and (c), the intervening medium is pure water. In (b) and (d), the medium is 10 mM TRIS buffer, at pH 7.4.

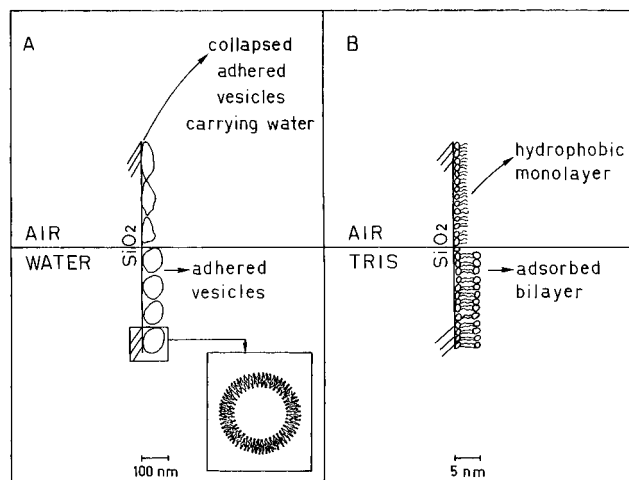


Figure 5. Models for the DODAB assembly at the SiO₂ surface in air or under water when the intervening medium mediating the vesicle/surface interaction is water (A) or 10 mM TRIS buffer at pH 7.4 (B).

deposition at limiting adsorption only for the second experimental condition.¹⁴ Indeed, the amount corresponding to limiting adsorption in TRIS fairly agrees with the one expected for bilayer deposition whereas, in water, limiting adsorption stays well below figures consistent with one bilayer deposition.¹⁴

Although dynamic contact angle measurements can provide sound information as indeed shown here, additional information obtained with other methods should be kept in perspective (e.g., electrokinetic characterization with electroosmosis, pinhole estimation with ESCA, adsorbed amount determination with ellipsometry, film structure with Brewster angle microscopy, atomic force microscopy, etc.). Nevertheless, because the main force driving DODAB deposition as vesicles or as one entire bilayer is the electrostatic attraction between the negatively charged mineral surface and the cationic polar head of the amphiphile, it is reasonable to assume a close analogy for DODAB adsorption on spherical silica particles and on the less charged flat surface of the silicon oxide obtained by thermic

oxidation. This reasoning supports the correlation between DODAB adsorption isotherms on silica and the pictures proposed in Figure 5 on basis of the contact angle data.

Another interesting result is the bell-shaped profile obtained for the angle dependence on DODAB concentration, C (Figure 3b,d). At intermediate C , there is vesicle adhesion and fusion with bilayer deposition (maximal hydrophobicity). At large C , bilayer deposition is followed by further vesicle adhesion (decreased hydrophobicity). Bilayer deposition followed by further vesicle adhesion was previously described for DODAB adsorption onto oppositely charged latex particles.⁵ This would cause a decrease of the contact angle since once again adhered collapsed vesicles that carry water would decrease surface hydrophobicity. Most importantly, *a maximum in the contact angle curve defines the optimal DODAB concentration for bilayer deposition at 2 h of interaction time.*

The effect of interaction time on the contact angle is in Figure 4. In water, the angle attained at 15 min of interaction time is smaller than the one obtained for the bare surface and remains constant as a function of the interaction time. Further increasing the interaction time does not affect this constant angle (Figure 4a). In TRIS, a constant angle larger than the one for the bare surface is attained at 100 min (Figure 4b). This angle can be taken as an equilibrium contact angle, allowing calculation of a mean value and its mean standard deviation. The error bars in Figure 3 correspond to mean standard deviations for measurements taken after equilibrium was attained at 1.5 h interaction time.

Dynamics of Vesicle/Surface Interaction from Determination of Dynamic Contact Angles. It is interesting how dynamical aspects of the vesicle/surface interaction can be further elucidated from the point of view of the dynamic contact angle (Figure 4). In water, equilibrium angles are smaller than those obtained for the bare surface, suggesting a final configuration of adhered vesicles on the surface (Figure 4a). In water, vesicle adhesion took place over the first 15 min of the interaction (Figure 5A). In TRIS, the same occurs over the first 15 min, but from 20 min onward, the angle steadily increases up to equilibrium at a much more hydrophobic surface coverage (Figure 4b) as in the picture in Figure 5B. The dynamic contact angle technique powerfully describes vesicle adhesion against bilayer deposition along time at a given C .

Regarding hysteresis, in water, advancing and receding angles differed by about 20° (Figure 4c and Figure 3c). However, at bilayer coverage in TRIS (at maximal angle in Figures 3b and 4b) hysteresis not only drops considerably but also comes to values very close to zero. At maximal angle, advancing and receding angles differ at most by ±5° largely inside the experimental error of the technique (Figures 3d and 4d).

For DHDAA interacting with mica sheets, MacGuiggan and Pashley²⁶ also observed that their receding angles were usually smaller than the corresponding advancing ones, though their hysteresis was larger than ours (20° or more for the difference between advancing and receding angles). Possibly, observation of hysteresis is related to desorption of the amphiphile from the surface. (One should recall that DHDAA is more soluble than its C18 counterpart.) Since the solubility of our vesicle-forming DODAB is very low, in the present work hysteresis due to desorption would be almost absent.

For poly(styrene/methacrylate) copolymer rods interacting with DODAB, the contact angle increased as a function of the amphiphile concentration, attaining a plateau value that depended on the charge density of the polymer surface.¹⁰ At the plateau, there was no hysteresis for DODAB dispersions

interacting with the most charged poly(styrene/methacrylate) rods.¹⁰ The same was observed for DODA chloride and DODA acetate.¹⁰ Thus, two completely different materials such as the copolymer¹⁰ and the mineral oxide (Figures 3d and 4d) yielded a minimum in contact angle hysteresis upon coverage with a DODAB bilayer.

Contact angle hysteresis has also been related to mechanical and/or chemical surface heterogeneities. If surface roughness were the major factor accounting for hysteresis, coverage with one bilayer would not be expected to effectively reduce surface roughness, and thereby bilayer coverage would have no effect on the hysteresis measured. On the other hand, chemical heterogeneity would indeed be eliminated by covering the surface with a chemically homogeneous DODAB bilayer. Thus, chemical heterogeneity on the SiO₂ surface is the most probable cause for contact angle hysteresis observed for the bare surface. This conclusion fairly agrees with detailed work in the literature regarding polishing effects on the bare SiO₂ surface.²⁸ A remarkable decrease in contact angle hysteresis (from 50° to 10°) was obtained by chemomechanical polishing, a procedure known to eliminate chemical heterogeneity on the native surface. Considering the limits of the experimental error, an even more remarkable effect is described here: the reduction of contact angle hysteresis practically to zero by means of bilayer coverage.

Conclusions

Cationic DODAB vesicles in the gel state may cause changes in wettability of planar SiO₂ surfaces due to vesicle adhesion or bilayer deposition. Depending on ionic strength being 0 or 10 mM monovalent salt, vesicles either adhere or open up upon contact with the surface. Bilayer deposition typically corresponds to absence of contact angle hysteresis and maximization of contact angles. The results for planar SiO₂ surfaces agree with previously reported adsorption isotherms for DODAB adsorption on silica in water or in 10 mM monovalent salt.¹⁴

Acknowledgment. We thank FAPESP and CNPq for financial support. L.C.S. wishes to thank CAPES for a fellowship as well as Mr. Zambom and Mr. Mansano for their help. The use of facilities at the Departamento de Engenharia Eletrônica—LSI-Escola Politécnica da USP is gratefully acknowledged.

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