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Atomic Force Microscopy Imaging of Substrate and pH Effects on Langmuir–Blodgett Monolayers

R. Viswanathan, D. K. Schwartz, J. Garnaes, and J. A. N. Zasadzinski*

Department of Chemical and Nuclear Engineering, University of California, Santa Barbara,California 93106

The atomic force microscope (AFM) provides a nondestructive way to image Langmuir–Blodgett (LB) films to determine film quality at length scales from nanometers to micrometers. Monolayers of cadmium arachidate deposited on mica and silicon at pH values of 5.5 and 6.5 were imaged by the AFM. Holes 10–100 nm in diameter were present in the monolayer on mica made at a pH of 5.5. The depth of the holes was roughly 3 nm, corresponding to the thickness of a monolayer. However, an image of a monolayer deposited on mica at a subphase pH of 6.5 yielded a uniform surface, although the film was more easily damaged by the AFM than the pH 5.5 monolayers. Monolayers on polished silicon wafers with native oxide were homogeneous and hole-free for films made at pH 5.5 and 6.5. The differences in film quality were ascribed to the less extensive hydration of the mica at pH 5.5, with secondary effects due to the pH-dependent complexation of cadmium with the arachidic acid. The microscopic roughness of the monolayers was not measurably different from that of the substrates. No lattice structure was observed for the monolayers, regardless of substrate, suggesting a disordered, fluidlike surface.

Introduction

The atomic force microscope (AFM) provides new opportunities for studying the organization and structure of monolayers and bilayers of phospholipids, proteins, lipoproteins, etc., at the molecular level.[1] Unlike its predecessor, the scanning tunneling microscope (STM), the AFM was designed to image nonconductive surfaces,[2] including such soft surfaces as biological cells and membranes.[3] Although the forces between tip and sample are on the order of 10^{-8} N when imaging is done in air, these forces are applied over exceedingly small areas, resulting in large localized pressures.[1] Most organic or biological samples cannot withstand such pressures without suitable modification. Langmuir–Blodgett (LB) deposition can immobilize such samples in a well-ordered way at a prescribed orientation to create model monolayers and bilayers for examination by AFM. We show here that the AFM is also the ideal tool for investigations of film quality and substrate–film interactions for LB monolayers.

Langmuir–Blodgett films are formed by transferring molecular monolayers of amphiphilic molecules from a liquid surface onto a solid substrate.[4] These films are models for biological systems such as cell membranes, which are bilayers comprised of amphiphilic molecules, along with proteins, cholesterol, etc. Depending on the deposition, either the polar headgroups or the nonpolar hydrocarbon side of the monolayer can be examined.[5] Hence, studying LB films has great significance in biology and medicine, as well as applications in biosensors, optics,

and nanolithography.[5,6] As LB films are quite fragile, besides being of molecular dimensions, sensitive but nondestructive techniques are needed for determining film quality. The structure of LB films has been studied by electron imaging and diffraction,[7] X-ray diffraction,[8] and scanning tunneling microscopy,[9,10] as well as by various spectroscopies.[11,12] Recently, atomic force microscopy has been used to image defects as small as a few nanometers wide in monolayers of cadmium arachidate on mica, although the origin of these defects was not determined.[5]

In this study, we continue our investigation of defects in LB monolayers by examining the effects of substrate and pH on monolayer quality. Divalent metal ions, such as cadmium, in the subphase have often been linked to the quality and stability of LB films of fatty acids. The amount of Cd^{2+} adsorbed at the substrate surface can be varied by changing the pH of the subphase. We report images of LB monolayers deposited at a pH corresponding to a fully Cd^{2+} saturated fatty acid salt (pH 6.5) and a monolayer with approximately even proportions of fatty acid and salt on two common types of substrates, muscovite mica and polished silicon wafers covered with the native oxide. Our results suggest that the pinhole defects seen are due to the relative lack of hydration of mica at pH 5.5 in comparison to mica at pH 6.5[13] and silicon oxide at both pH's, or to a combination of this lack of hydration and the smaller amount of Cd^{2+} complexed with the arachidic acid at pH 5.5.

* To whom correspondence should be addressed.

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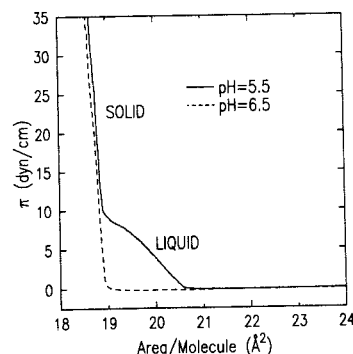
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Figure 1. Isotherms taken from slow compression (4 Å molecule^{-1} min^{-1}) of arachidic acid at the air–water interface of a NIMA Langmuir trough. The subphase contained 0.6 mM cadmium chloride; the pH was adjusted with NaHCO$_3$ to either pH 5.5 or 6.5. Note the presence of an intermediate, compressible or "liquid" phase region in the pH 5.5 isotherm, followed by a less compressible, "solid" phase at high surface pressure. The liquid phase is eliminated at pH 6.5 due to complexation of the arachidic acid with cadmium ions.

Materials and Methods

The LB films were spread at the air–water interface of a commercial NIMA[14] Langmuir–Blodgett trough. Arachidic acid (CH$_3$(CH$_2$)$_{18}$COOH, 99% purity) was dissolved at approximately 2 mg/mL in spectranalyzed chloroform[15] and deposited as small droplets on the air–water interface. The subphase water was treated in a Millipore Milli-Q water purification system.[16] Cadmium chloride (CdCl$_2$, 99.99% purity) was added at a concentration of 0.6 mM to the subphase. The pH of the CdCl$_2$ solution was 5.5. For some studies the pH of the subphase was adjusted to 6.5 by addition of sodium bicarbonate (NaHCO$_3$, 99.95% purity). Plots of surface pressure versus area per molecule are shown in Figure 1 for both pH's. At the lower pH, the appearance of a compressible liquid phase is indicative of un-complexed arachidic acid in the monolayer.

The mica substrates were attached to steel disks with epoxy. After ultrasonic cleaning with ethanol for 5 min, the mica was cleaved using ordinary adhesive tape, and inserted into the subphase for use in deposition. The silicon (100) substrates[17] were cleaned in a solution of 70% H$_2$SO$_4$/30% H$_2$O$_2$ at a temperature of 90 °C for 30 min and rinsed thoroughly with Milli-Q water. This procedure cleaned the substrate of organic impurities, leaving the amorphous native oxide intact. The substrates were either used immediately or stored in Milli-Q water (for less than 1 day) until deposition. Approximately 40 μL of the arachidic acid solution was deposited on a trough area of about 700 cm^2. After a wait of approximately 15 min to allow solvent evaporation, the film was slowly compressed to a pressure of 30 mN/m where feedback was engaged to keep the pressure constant during deposition. At this pressure the monolayer is in the "solid" or condensed phase independent of the subphase pH, with roughly the same area per molecule (See Figure 1). The trough area was monitored for the first 5 min of pressure control to ensure film stability. This was to ensure that any relaxation effects had completely ceased so deposition transfer ratios would not be affected. The resulting transfer ratios were in the range 0.95–1.05, indicating full monolayer coverage. Dipping speeds were approximately 2 mm/min.

After deposition, the monolayers were stored in air in a sealed container for no more than 2 days prior to imaging with the AFM. A Nanoscope II-FM AFM[18] was used in the so-called "height" mode (i.e., tip to sample distance is adjusted by feedback to keep the force constant), using microfabricated silicon nitride cantilevers[18] that were 200 μm long, 0.6 μm thick, and 36 μm wide, with a spring constant of 0.12 N/m. Imaging was done in air with a 15-μm × 15-μm scanner. Typical forces used were on the order of 10 nN. All images shown were stable for long periods and did not change with repeated AFM imaging, except as noted.

Contact angle measurements were performed using a custom-built apparatus. Pure Milli-Q water was applied to and withdrawn from the sample using a syringe to measure advancing and receding contact angles, respectively. The measurements were made through the use of an eyepiece with cross-hairs calibrated to degrees and minutes. Resolution was less than 1°.

Results

Figure 2a shows an AFM image of a cadmium arachidate monolayer transferred from a subphase with pH 5.5 onto mica. As is visible from the image, many holes are present in the film. The holes correspond to the dark areas of the image, with the lighter "background" being the surface of the film. In the 4-μm × 4-μm scan, there are approximately 6 holes/μm^2 (or about 90–100 holes in the entire scan). The larger holes are 100-200 nm (diameter) in size, while the smaller holes are 10–100 nm in size. A height measurement between a typical hole and the adjacent flat surface (Figure 2b,c) shows that these are indeed holes in the film. The measurement yielded a depth of 2.85 nm, approximately the thickness of a cadmium arachidate monolayer (about 3 nm). Subsequent images taken over the same range and at different locations on the film showed a similar density of holes. The holes invariably were circular, with no indication of a crystalline lattice in the monolayer. The images of the holes were readily reproducible without causing any appreciable damage to the sample. Roughly 6% of the scan area (16 μm^2) was covered with holes.

Figure 3 shows an AFM image of a cadmium arachidate monolayer on mica at a pH of 6.5. Unlike the lower pH trial, no holes are visible and the film appears relatively flat and featureless. The protrusion that appears in the film emerged after scanning for about 15 min. These structures increased with further scanning and are presumably related to gradual destruction of the film by the AFM tip. Images of this type of film were obtained only by use of extremely small forces and often degraded with time. In Figure 3, the central area showing protrusions had been scanned for 20 min before "zooming" out for this image. By increasing the applied force, we could pierce through the film to image the 0.5-nm hexagonal lattice of the mica substrate.[5] Again, height measurements done between the newly-scraped hole and the surrounding film yielded a value around 3 nm. Increasing the applied force to pierce through the film to the substrate could be done on all of the monolayers studied in these experiments, for both substrate verification and film thickness measurements.

LB film quality was verified through contact angle measurements done on the monolayers deposited on mica. These measurements yielded advancing contact angle values of 94.5° ± 2° for the higher pH film and 85.6° ± 5° for the lower pH film. When compared with literature values for various LB films, the higher pH film value was consistent with the higher contact angle values reported (between 90° and 100°) while the low pH film represented the lower reported values (≤85°).[19-21] The measured

(14) NIMA Technology, Ltd., Warwick Science Park, Coventry CV4 7EZ, England.

(15) All of the chemicals used in this study were of the highest available purity. Arachidic acid (eicosanoic acid) and sodium bicarbonate were purchased from Aldrich Chemical Co., Milwaukee, WI. Cadmium chloride was purchased from Sigma Chemical Co., St. Louis, MO. Spectranalyzed chloroform was purchased from Fisher Scientific, Santa Clara, CA.
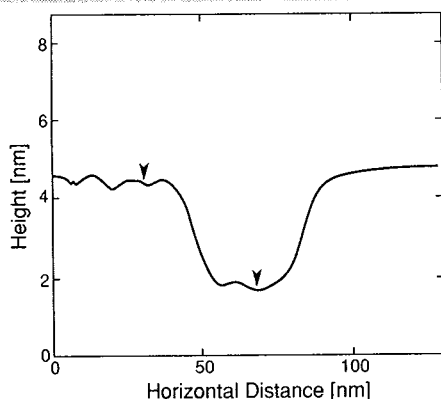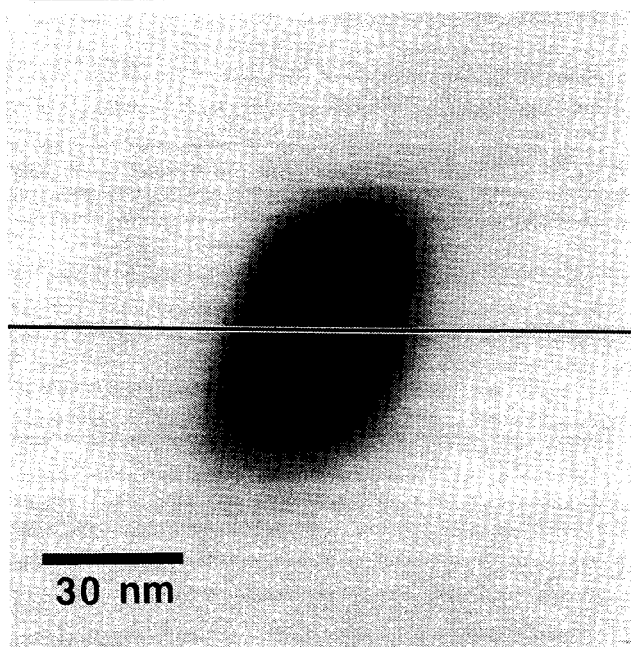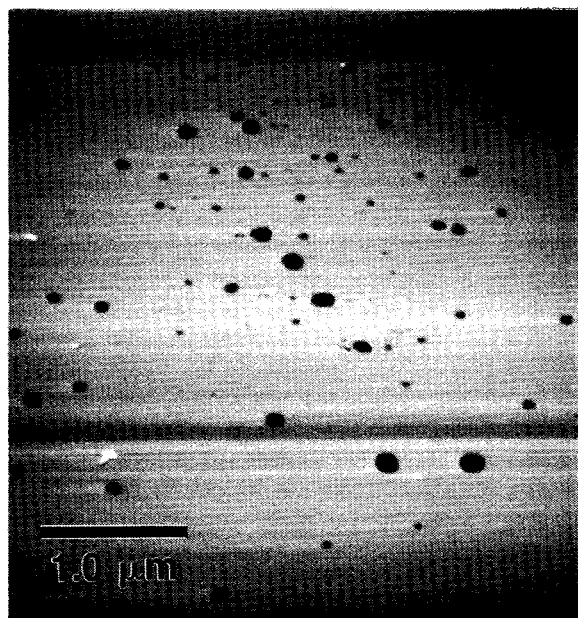
(16) Millipore Corp., Bedford, MA.

(17) The polished silicon wafers (orientation (100), 3 Ω cm, n-type) were purchased from Semiconductor Processing, Boston, MA.
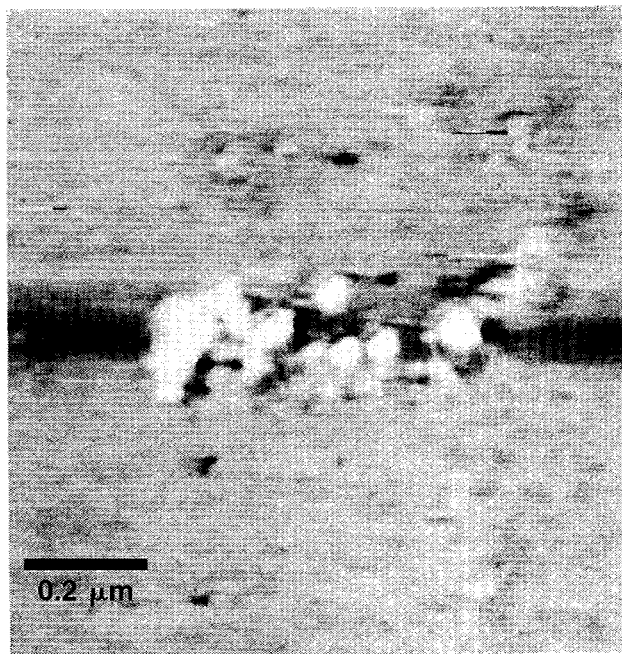
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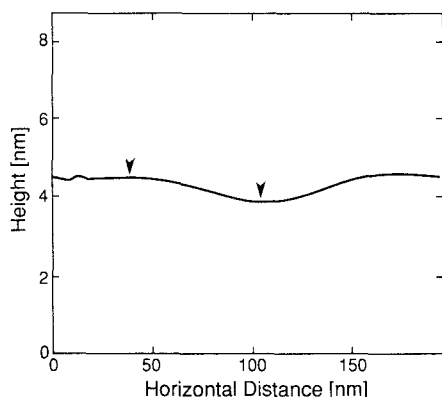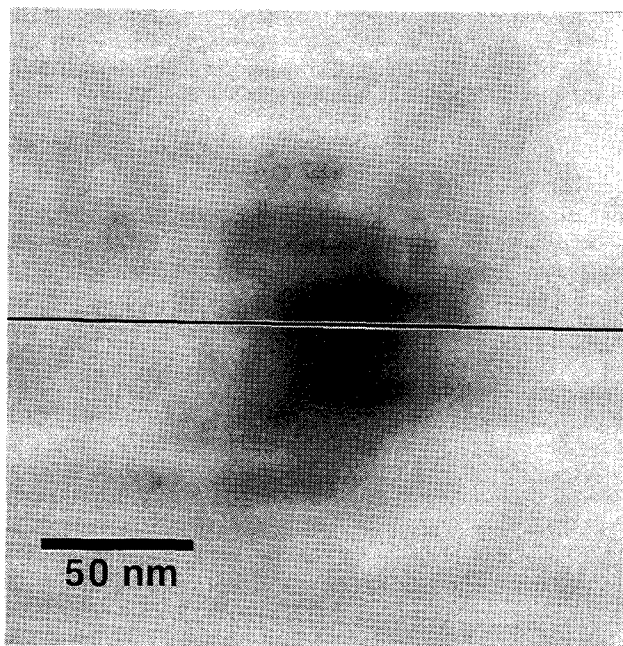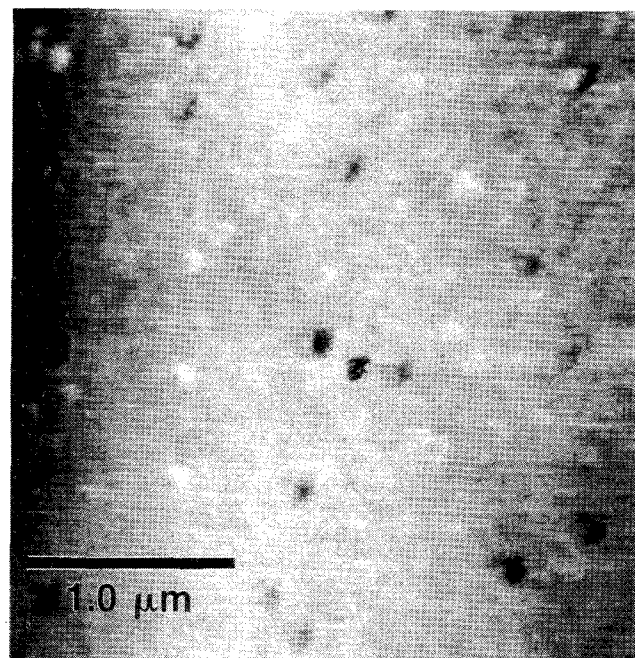(20) Tadros, M. E.; Hu, P.; Adamson, A. W. *J. Colloid Interface Sci.* **1974**, *49*, 184.

Figure 3. AFM image of a monolayer of cadmium arachidate deposited on mica at a pH of 6.5. The image size is 1000 × 1000 nm. The surface appears to be quite smooth and relatively devoid of holes, unlike the low pH on mica case (Figure 2). The protrusion visible on the film emerged after about 15 min of imaging, presumably from contamination that the AFM tip had picked up during rastering and deposited on the film. The approximately 1-nm thickness of the protrusion suggests that this is not a second layer, which would be about 3 nm in thickness.

contact angles on the films deposited on mica serve to confirm the conclusions made from the AFM images. The higher contact angles of the films deposited at pH 6.5 imply a more perfectly ordered and thus more hydrophobic surface. Lower contact angle values reported in the literature[19-21] are often associated with holes or defects as in the film deposited at pH 5.5. Receding contact angle measurements made for both films yielded values approximately 5° lower than the corresponding advancing values.

Figures 4a and 5 show AFM images of cadmium arachidate monolayers deposited on silicon at pH's of 5.5 and 6.5, respectively. As can be seen from the pictures, both images are similar and show a flat and homogeneous film. No holes initially existed, and the monolayers were smooth. However, when the applied force was increased slightly, holes were readily scraped in the films by the AFM tip. Thus, although the films on silicon were flat and homogeneous, they were fragile and subject to damage by the AFM tip, as was the case with the high-pH film on mica. As can be seen from comparing Figures 4a and 5, the low-pH monolayer on silicon is a bit rougher than its higher pH counterpart. The rms roughnesses for the pH 5.5 and 6.5 films were 0.16 and 0.11 nm, respectively. However, from a height measurement done across a small portion of the lower pH film, shown in Figure 4b,c, it is obvious that the extra roughness of the lower pH monolayer is due to surface asperities rather than holes in the monolayer. Height measurements gave variations of 0.2–0.6 nm with an average asperity depth of 0.38 nm, far from the value for a monolayer thickness (~3 nm).

When imaging is done with the AFM, it is not uncommon to see a hole in the center of the image. This results from the fact that the initial approach of the tip to the surface

Figure 2. (a) AFM image of a monolayer of cadmium arachidate deposited on mica at a pH of 5.5. The image size is 4000 × 4000 nm. Note the presence of many irregular, circular holes in the film, varying in size from 10 to 200 nm across. These holes, appearing dark in the image, correspond to lower regions while the monolayer is light, corresponding to higher regions. The depth of the holes is approximately 3 nm. (b) Small zoomed portion of the same film shown in (a) with (c) a height profile showing the thickness of a typical hole to be 2.85 nm, consistent with the thickness of a monolayer. The image size is 150 × 150 nm.

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Figure 5. AFM image of a monolayer of cadmium arachidate on silicon (100) done at a pH of 6.5. The image size is 4000 × 4000 nm. The film is extremely smooth and homogeneous throughout, even more so than the monolayer on silicon at pH 5.5 (Figure 4a). As in all of these films, increasing the applied force resulted in the AFM tip piercing the monolayer, creating a hole approximately 3 nm deep, corresponding to the thickness of a monolayer.

can result in much larger forces than those used for imaging. These capillary forces come about from the surface tension that results from liquid bridges forming between the tip and the sample.[5] This destructive technique, although allowing for a way to verify the presence and thickness of a monolayer, can be circumvented by reducing the force and reimaging over a different scan area.

Discussion

The results of these experiments verified the potential of the AFM as a tool for determining LB film quality and for imaging defects. These results can be used to determine whether cadmium arachidate monolayers can be used in various applications, such as serving as anchors or substrates for preparing lipid bilayers. We have also used the results of this study as a feedback to optimize the parameters of LB film deposition, leading to more perfect LB monolayers and multilayers.

The detailed interpretation of these observations is complicated by the fact that the pH of the subphase has two independent effects. Changing the pH from 5.5 to 6.5 increases the amount of cadmium ion complexed with the fatty acid, and (in the case of mica) it affects the degree of hydration of the substrate at equilibrium. It has been shown[22,23] that the amount of Cd^{2+} present at the arachidic acid–water interface is quite sensitive to pH in the range of 5–7, with approximately 50% cadmium complexation at pH 5.5 and essentially full complexation at pH 6.5. By coordinating with two carboxylic acid groups, the Cd^{2+} acts to dimerize the arachidic acid. The cadmium arachidate thus formed begins to associate at low surface pressures to form islands of an ordered phase.[7,8] This is manifested in the isotherms (Figure 1) by the absence of a "liquid" or compressible phase as the area per molecule is decreased.

Figure 4. (a) AFM image of a monolayer of cadmium arachidate on silicon (100) done at a pH of 5.5. The image size is 3000 × 3000 nm. The asperities on the surface are approximately 0.2–0.6 nm deep and do not appear to be holes in the monolayer. The surface is still relatively smooth and homogeneous. (b) Small zoomed portion of the same film shown in (a) with (c) a height showing a fairly flat film. The depth of the asperity is 0.61 nm, confirming it to be an asperity and not a hole in the monolayer. The image size is 200 × 200 nm.

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In addition to the pH effects on the monolayer, there is also a pH effect on the mica substrate. It is well established that clean silicon oxide surfaces are hydrated due to short-ranged hydration forces[24-28] at both pH 5.5 and 6.5. Riegler and Legrange show that the work of adhesion of a LB film on silicon is independent of pH from 5.5 to about 9.[24] On the other hand, Pashley and Israelachvili report that the pK_H of mica is 6.0 and that the extent of hydronium ion adsorption to mica controls the extent and range of hydration forces.[29] At lower pH, the adsorption of divalent ions to mica is also reduced.[29] Hence, mica in acidic solutions has little or no hydration and small or absent hydration forces,[13] and the degree of hydration should change dramatically between the pH values of 5.5 and 6.5. As a consequence, the amount of water that is associated with the substrate and the polar region of the monolayer is likely to be much less for a monolayer deposited on mica at pH 5.5 than for a monolayer deposited on silicon oxide or on mica at pH 6.5.

During deposition onto a silicon oxide substrate, there is a thin layer of water present between the film and the substrate, which likely remains even after equilibrating in air with typical humidities of 50–70%.[24-28] It is likely that this hydration layer allows the monolayer to retain more of the character of the monolayer at the original air/water interface, in particular its packing density. Merkel et al. have also shown that the presence of a thin water layer between the substrate and the headgroups of the monolayer increases the lateral diffusivity of the molecules in the monolayer.[30] This additional mobility of the monolayer[30] might allow defects that would otherwise form to anneal more rapidly than in the absence of the water film, especially in the case of the less homogeneous arachidic acid/cadmium arachidate mixed monolayer formed at lower pH. At pH 6.5, the mica substrate is like the silicon in that it should be hydrated at equilibrium. However, at pH 5.5 the hydration layer is likely to be absent due to the lack of hydration forces. The lack of a hydration layer must, therefore, be an important factor in causing the holes we see in the film deposited on mica at pH 5.5. However, as of yet, we cannot rule out the possibility that the smaller amount of cadmium present also plays a role,

even though it does not have much effect on the more hydrated silicon substrate.

At present, it is not known whether holes are formed during deposition or if they form upon "drying" of the film in air. To test this, we plan a set of experiments to examine the effects of humidity and aging in different environments to examine annealing in these settings. For example, a monolayer on silicon can be placed in a desiccated environment to see whether holes emerge (due to evaporation or dehydration of the hydration layer), and a monolayer made at pH 5.5 on mica could be placed in a fully saturated environment (humidity 90–100%) to see whether the holes close up due to a possible formation of a hydration layer.

Even in the films without holes, the AFM could be used to give height measurements by purposely increasing the force and measuring the depth of the holes that result.[5] The ability to make such holes in LB films and image them with the AFM raises new opportunities in various applications such as nanolithography and high-density permanent information storage.[5] The results of this study demonstrate the varying effects of pH and substrate on film quality. They confirm that the AFM can nondestructively determine LB film quality with subnanometer resolution, and the importance of substrate on film quality.[5] Electron microscopy techniques for examining film quality are limited to specific substrates and cannot resolve the defects shown here.[31-34]

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