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Structural Studies of Aliphatic Substituted Phthalocyanine−Lipid Multilayers

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A Langmuir−Blodgett film of aliphatic substituted phthalocyanines on a C18 silane supporting layer coupled onto a silicon substrate has been investigated using neutron reflectometry. This multilayer structure is seen as a possible candidate for phthalocyanine−lipid biosensor devices. The results show the suitability of the C18 ligands as an anchoring layer for the phthalocyanines. The scattering length density profiles demonstrate the effectiveness of a lipid monolayer in partitioning the composition of phthalocyanine layers from that of the bulk liquid. The effectiveness of this barrier is a critical factor in the efficiency of such devices.

Introduction

Since the discovery of phthalocyanines (Greek word, rock oil blue) in 1907[1] and their subsequent in-depth structural and chemical study a number of years later,[2] these compounds have shown potential in countless applications. Phthalocyanines are thermally and chemically very stable. They also display intense color and possess catalytic, electrical, as well as photophysical properties.[3] Phthalocyanine derivatives can form Langmuir monolayers at the air−water interface. Their deposition on a solid support using the Langmuir−Blodgett (LB) technique on hydrophobic surfaces has shown that the phthalocyanine ring will exhibit an edge-on orientation.[4] This is thought to be due to the amphiphilic character of these compounds.

The design of integrated optical sensors using aliphatic substituted phthalocyanines for continuous monitoring and spot checks on water quality has been reported.[5] These novel and highly sensitive, integrated optical biosensors are based on detection of change in the refractive index of a sensing layer saturated with nitrous molecules encapsulated between a biomimic membrane (phospholipid) and a solid support. The change in refractive index is measured using a stacked planar waveguide in a dual-polarization interferometer arrangement in these devices. The diffusion of entrapped nitrous molecules through the biomimic encapsulation will change as a function of damage caused by either bioagents (bacteria, viruses) or chemical agents (heavy metal contamination, pesticides) and is detected by change in the refractive index of the sensing layer.

The focus of our project was to study the structure and hence optimization of the design of a disposable version of these sensors, removing the need for optical measurement and the reliance on the waveguide as a means of detection. This design will involve replacing the optical waveguide part of the sensor by a multilayer of an aliphatic substituted phthalocyanine compound, which can incorporate a color pigment, sensitive to nitrous oxide, which is in turn deposited on a hydrophobically modified silicon substrate using the Langmuir−Blodgett technique. A schematic diagram of such a device is shown in Figure 1. The first step in the commercialization of these devices involves a better understanding of the structure of the interfaces, that is, layer uniformity, the composition, and the effectiveness of a lipid layer as a membrane separating the sensing region from that of the bulk water. We report here a structural study of Langmuir−Blodgett deposited aliphatic (C10) substituted phthalocyanines (Figure 2) and lipid multilayer structures on a hydrophobic solid support using neutron reflectometry technique. We seek to determine the water content of the phthalocyanine multilayers, as this plays an important role in the long term stability of these devices, and also to investigate the interface between the aliphatic (substituted phthalocyanine molecules) and the mimic layer (lipid monolayer). The results will provide a better understanding of the interfaces and will ultimately enable engineering of more robust and reproducible commercial biosensors.

Experimental Section

Materials. *d*-1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and functionalized poly(ethylene glycol) (PEG) lipids DSPE-PEG(2000) were purchased from Avanti (Avanti, Polar Lipids, Inc.). The synthesis of the aliphatic substituted phthalocyanine is reported elsewhere.[6] The D_2O was obtained from Fluorochem (>99 at. D%), and ultrapure H_2O was produced using an Elgastat water purification unit. The silicon blocks (Crystran Ltd., Poole, U.K.), used as the substrate, were circular in section with a diameter of 100 mm and a thickness of 10 mm and were polished on one (111) face to ca. 6 Å. The silicon surface was initially cleaned using the RCA method.[7] The substrates were then

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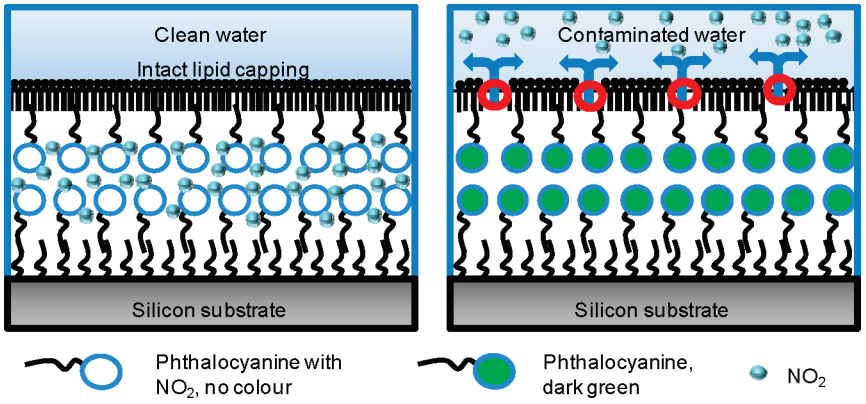

Figure 1. Schematic diagram of the phthalocyanine sensor device.

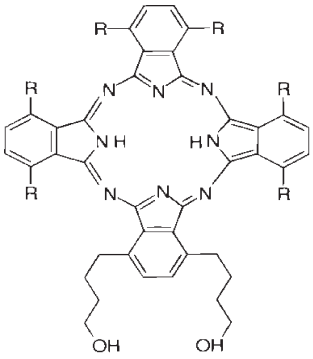

Figure 2. Chemical structure of the phthalocyanine ligand. The six R groups are $C_{10}H_{21}$.

chemically dried prior to treatment with a silane coupling agent octadecyltrichlorosilane (ACROS, 95%) using established methods.[8] This has been shown to provide a chemically bound hydrophobic layer on the silica surface.[9] In our previous work, we have characterized this layer by measuring the reflectivity from the Si−water interface using a series of contrasts.[9]

Neutron Reflectometery. Reflectivity measurements were carried out using the reflectometer SURF[10] at the ISIS Spallation Neutron Source, Rutherford Appleton Laboratory, Didcot, U.K. The neutron beam is polychromatic with wavelengths in the range $0.53 < \lambda < 6.9$ Å. To obtain the widest amenable momentum transfer, Q, range, reflectivity spectra were measured for a series of grazing incidence angles θ, where θ is the angle of incidence at the Si−water interface and $Q = (4\pi \sin \theta)/\lambda$ is the momentum transfer at the interface. The nominal incidence angles used were 0.35, 0.8, and 1.8°. The actual angles of incidence were determined by performing detector angle scans in reflection geometry once the height alignment of the sample with respect to the neutron beam has been optimized. The collimating slit settings were varied with incidence angle in order to measure all reflectivities with a constant angular resolution ($\delta\theta/\theta = 3\%$). The sample was under-illuminated with an illuminated length of ~45 mm projected on the sample.

The measured reflectivity profiles are normalized relative to the incidence beam monitor spectrum and corrected for detector efficiency as per standard reflectivity measurement on a time-of-flight instrument,[11] and the data were subsequently corrected for the wavelength-dependent transmission through the silicon substrate. The data obtained were then overlapped in Q.

Neutron reflectivity is a technique sensitive to the average neutron refractive index, n, profile normal to an interface.[8] The dispersive refractive index can be written as

$$n(\lambda) \approx 1 - \frac{\lambda^2}{2\pi} Nb + i\frac{\lambda}{4\pi} N\sigma$$

where λ is the neutron wavelength, $Nb = \Sigma_i N_i b_i$, and $N\sigma = \Sigma_i N_i \sigma_i$, with N_i being the number density, b_i the coherent scattering length, and σ_i the absorption and incoherent cross-section of nucleus i. The multiple Nb is known as the scattering length density of a medium with refractive index n. According to the above equation, the large difference in the scattering lengths of ^{1}H ($b = -3.7406$ fm) and ^{2}H ($b = 6.671$ fm) can be exploited in hydrogenous systems. Since Nb is linearly related to the volume fraction composition ($Nb \approx \Sigma_j \phi_j \overline{Nb_j}$, where ϕ_j is the volume fraction and $\overline{Nb_j}$ is the scattering length density of species j), a layer model with discrete strata representing regions with different chemical composition can be constructed and the reflectivity from such a model can readily be calculated. Each layer, i, has a thickness, d_i, refractive index, n_i, scattering length density, Nb_i, and interfacial roughness, σ_i. The reflectivity can be calculated using, among other methods, the standard optical-matrix method, and the parameters of the proposed layer model can be optimized using nonlinear least-squares fitting.

Two silicon blocks, S1 and S2, were used in these experiments. The surfaces of both these substrates were first cleaned and then rendered hydrophobic by means of silane coupling. A C-18 layer was deposited. A series of 2, 4, and 6 layers of substituted phthalocyanine were subsequently deposited onto the C18 coated silicon substrate S1, using Langmuir−Blodgett dipping technique.[12] The deposition was performed using a standard Teflon Langmuir trough. The trough was filled with ultrapure water (Milli-Q). A solution of phthalocyanine in chloroform was prepared and then carefully spread at the air−water interface. An ordered Langmuir film was then obtained by moving the poly(tetrafluoroethylene) (PTFE) barriers of the trough until a surface pressure of 25 mN m^{-1} was reached. The surface tension was monitored by the Wilhelmy plate method. A sample holder was manufactured to dip the silicon block into the water, and while the block was immersed, the surface pressure was maintained at 25 mN m^{-1} by computer controlled motion of the PTFE barriers. The sample holder allowed dipping/removing of the block from the aqueous phase at a constant speed of 5 mm/min. First, a bilayer was deposited. The block with a bilayer was then characterized using neutron reflecotmetery with D_2O forming the aqueous

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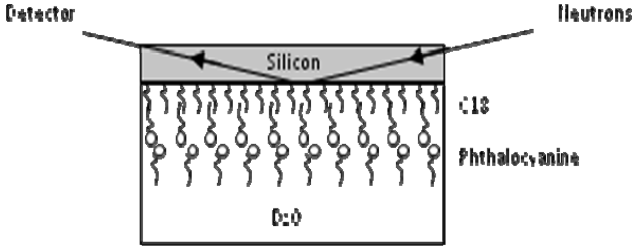

Figure 3. Neutron reflectometery experimental arrangement.

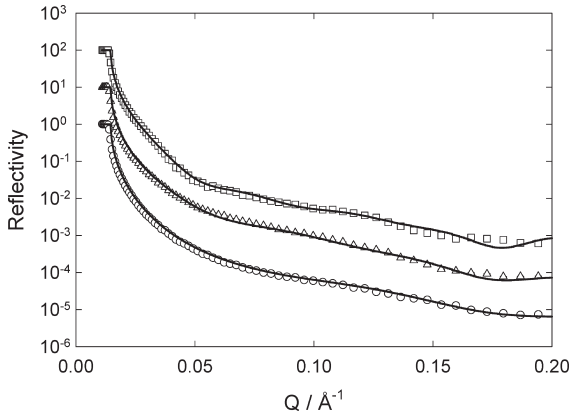

Figure 4. Reflectivity profiles for 1× bilayer (○), 2× bilayer (△), and 3× bilayer (□) of phthalocyanine at the silicon/D_2O interface. The solid lines show the best fit to the data. The profiles are shifted by a factor of 10 for the purpose of clarity.

subphase. After this first measurement, the block was carefully removed from the cell and a second bilayer was deposited using the same procedure described above. Now those two bilayers of phthalocyanine were again characterized using neutron reflectometery. The same procedure was followed for the deposition of the third bilayer.

In a second experiment, a mixture of DPPC and DPPE carrying a PEG group (3%w/w) monolayer were transferred on top of the second C18 coated second silicon substrate, S2, as a control experiment. The deposition of the lipid mixture was also performed at a constant surface pressure of 25 mN m^{-1} and a dipping speed of 5 mm/min. As only a monolayer had to be deposited, the block was immersed in the aqueous phase, and then the air−water interface was carefully cleaned by vacuum suction. Only then the block was slowly removed from the trough. The structure of this controlled monolayer was then determined. The lipid monolayer was then washed away, and a bilayer of phthalocynaine followed by a lipid monolayer was then deposited. In actual devices, the nitrous oxide would be entrapped below this lipids monolayer. A series of neutron reflectivity profiles were then obtained to characterize these layers.

The C-18 layer has already been characterized using a series of solvent contrasts on numerous occasions.[9] The silicon oxide layer and the C-18 hydrophobe layer were also characterized using the technique of ellipsometry (see the Supporting Information). The layer thickness and density of the oxide layer and C18 layer were then used as a starting point in our subsequent fitting procedure. The neutron reflectometery's experimental arrangement is shown in Figure 3.

Results and Discussion

The measured reflectivity data for the C18 coated silicon block S1 with 1, 2, and 3 bilayers of aliphatic substituted phthalocyanine with D_2O forming the aqueous subphase are shown in Figure 4. The solid lines correspond to modeled reflectivity based on the

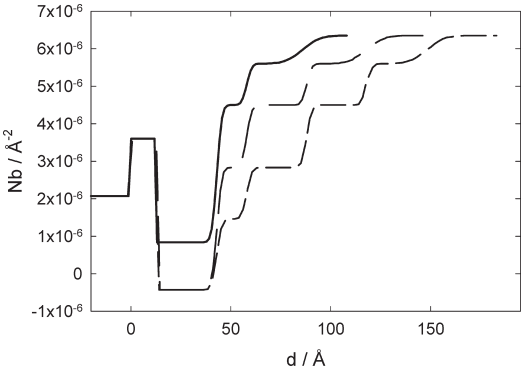

Figure 5. Scattering length density profiles for 2 (solid), 4 (long dash), and 6 (short dash) layers of phthalocyanine at the silicon−D_2O interface.

Table 1. Fitted Structural Parameters Obtained from the Fits to the Reflectivity Profiles Shown in Figure 4

	Si (S1) substrate			
	layer	layer thickness (Å)	NB ($\times 10^{-6}$ Å^{-2})	roughness (Å)
		1× bilayer phthalocyanine		
	C18	30 ± 2	0.84	2
1	phthalocyanine	15 ± 2	4.50	2
2	phthalocyanine	28 ± 2	5.60	7
		2× bilayer phthalocyanine		
	C18	30 ± 2	−0.43	2
1	phthalocyanine	15 ± 1	2.83	2
2	phthalocyanine	30 ± 2	4.50	2
3	phthalocyanine	30 ± 2	5.60	7
		3× bilayer phthalocyanine		
	C18	30 ± 2	−0.43	2
1	phthalocyanine	15 ± 2	1.46	2
2	phthalocyanine	30 ± 2	2.83	2
3	phthalocyanine	30 ± 2	4.50	2
4	phthalocyanine	30 ± 2	5.60	7

scattering length density profiles that are shown in Figure 5. In the modeling of the data, the thickness and scattering length density of the oxide film was found to be $d = 13 \pm 2$ Å, $Nb = 3.6 \times 10^{-6}$ Å^{-2}. The fitted structural parameters obtained from these fits are given in Table 1.

The C18 layer was found to be 30 ± 2 Å, which is slightly thicker than the expected value of 24 Å reported previously[9] and a value of 17 ± 2 Å for a dry C18 layer coupled silicon sample deduced from the ellipsometry data. This indicates an entanglement of the C18 chain with the aliphatic tail of the phthalocyanine layer, hence the good anchoring characteristic of the C18 Layer. Neutron reflectometery cannot distinguish between the C18 chain and the aliphatic tail of the phthalocyanine because of the lack of contrast between the protonated C18 and the aliphatic part of the phthalocyanine which is also protonted (similar scattering length density value). One striking feature of these results is the fact that as the number of phthalocyanine bilayers increases, the water (D_2O) content of the underlying structures decreases. This can be seen in the scattering length density profiles (Figure 5). The total layer thickness for the aliphatic substituted phthalocyanine for the 1 bilayer sample was found to be 43 Å, for the 2 bilayers 75 Å, and for the 3 bilayers 105 Å. This indicates a 13% and an 18% reduction in the overall ideal layer thickness for 2 and 3 bilayer samples which results from the D_2O expulsion as more layers are

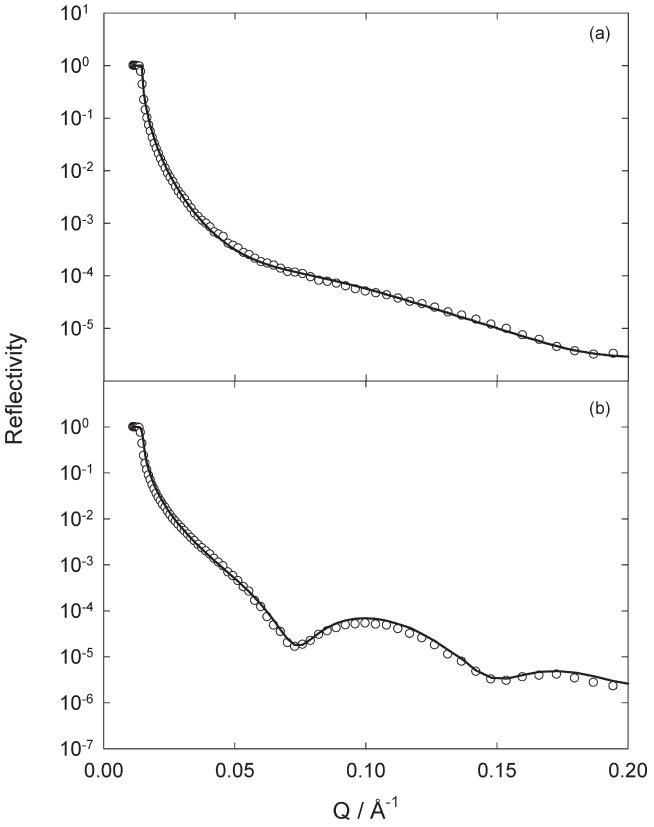

Figure 6. (a) Reflectivity profiles for DPPC-DPPE+PEG layer and (b) 2 layers of phthalocyanine covered by DPPC-DPPE+PEG at the silicon−D_2O interface. The best fits to the data are shown by solid lines.

Table 2. Fitted Structural Parameters Obtained from the Fits to the Reflectivity Profiles Shown in Figure 6

	Si (S2) substrate			
	layer	layer thickness (Å)	NB ($\times 10^{-6}$ Å^{-2})	roughness (Å)
	C18 + DPPC monolayer			
	C18 + *d*-lipid	19 ± 2	0.55	4
1	lipid-chain	16 ± 2	3.30	3
2	lipid head-PEG	60 ± 5	5.83	14
	C18 + 1× bilayer phthalocyanine + DPPC monolayer			
	C18	30 ± 2	−0.12	2
1	phthalocyanine	15 ± 1	1.22	2
2	phthalocyanine	30 ± 1	2.32	2
2	lipid-chain	16 ± 1	3.48	5
3	lipid head-PEG	60 ± 5	5.83	20

deposited. This is also supported by the scattering length density profiles (Figure 5).

In part one of the second experiment, a mixture of DPPC and DPPE carrying a PEG group (3%w/w) was deposited as a monolayer on a hydrophobic Si (C18 coated) surface (S2). The reflectivity profile is shown in Figure 6a. The fit to the data indicated a mixing of the deuterated tail of DPPC and the protonated C18 chain over a 19 Å region. This confirms the earlier results for the S1 sample (i.e., the interpenetrations of the aliphatic chains) and can now be seen because of better contrast. A three layer model was required to fit this data: a 19 Å layer consisting of a mixture of C18 and DPPC tail, a 16 Å layer representing the DPPC tail region, followed by a 60 Å layer

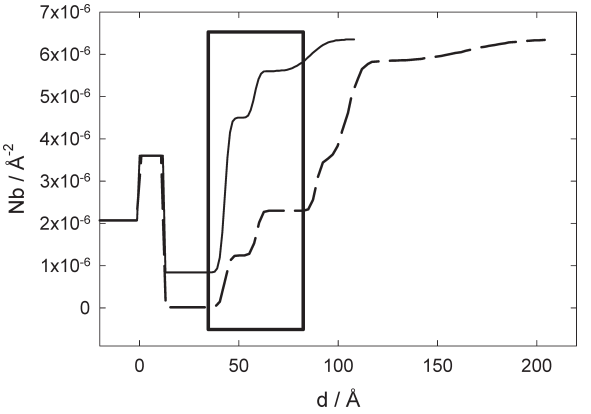

Figure 7. Scattering length density profiles for DPPC-DPPE+PEG layer (solid) and 2 layers of phthalocyanine covered by DPPC-DPPE+PEG (long dash) at the silicon−D_2O interface. The box represents the phthalocyanine layer contribution to the data.

representing the large PEG and DPPC headgroup region. The fitted parameters are given in Table 2.

The lipid monolayer was then washed from the S2, and a bilayer of phthalocyanine was deposited followed by a lipid mononlayer. The reflectivity data obtained are shown in Figure 6b. This deposited layer sequence represents an ideal device structure. The water penetration into the phthalocyanine bilayers was found to be less than that in the case of the 1× bilyaer phthalocyanine (S1 sample) with the same overall layer thickness. The water content of the phthalocyanine bilayer is an indication of the ability of the lipid monolayer to form an effective barrier between the D_2O bulk and the phthalocyanine bilayer beneath. These are ideal characteristics required for these types of devices. The full scattering length density profile for the fit is shown in Figure 7. The fitted structural parameters are given in Table 2.

Conclusions

In this experiment, we have shown the suitability of the C18 ligands as an anchoring layer for phthalocyanine on a solid support. The thicknesses of multilayers of phthalocyanine at the silicon−air interface measured with ellipsometry (see the Supporting Information) prior to the solid−liquid neutron experiment were 41, 82, and 124 Å, respectively, for 2, 4, and 6 layers, in overall good agreement with the neutron data and those reported in the literature.[13] Although the layer thicknesses reported here for neutron are slightly lower than these because of the interpenetrations of C18 and the aliphatic substituted phthalocyanine and the lack of scattering contrast between these layers.

The data for the phthalocyanine bilayer with the deposited lipid monolayer showed almost a complete depletion of water from the C18 layer in addition to a depletion of water from the phthalocyanine bilayer. This result demonstrates the effectiveness of the lipid layer in partitioning (sealing) the deposited phthalocyanine layers from the bulk water. This is crucial for viability of these systems as the biosensors' operation relies on the entrapment and subsequent release of nitrous oxide gas depending on the integrity of the lipid membrane. However, some trapped water content of these layers is required to maintain the sensing layer saturated with nitrous molecules for effective functioning of these devices. In a future experiment, we plan to use polished quartz as a substrate with color incorporated into the phthalocyanine bilayer. The transparent quartz will allows us to observe the color changes

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in the sensing layer when the lipid membrane is attacked, resulting in the escape of the nitrous oxide gas, while simultaneously measuring the changes to the layer structures using neutron reflectometery.

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Supporting Information Available: Ellipsometry data obtained at the air−water interface. This material is available free of charge via the Internet at http://pubs.acs.org.