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## Neutron reflection from a dimyristoylphosphatidylcholine monolayer adsorbed on a hydrophobised silicon support

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### Abstract

Neutron specular reflection has been used to study the structure of a monolayer of dimyristoylphosphatidylcholine (DMPC) deposited using the Langmuir-Blodgett technique onto a silicon oxide substrate. A self-assembled monolayer of octadecyltrichlorosilane with a deuterated alkyl chain (d-OTS) had been previously bonded onto this silicon oxide substrate which rendered it hydrophobic. In the system under study, the alkyl chains of the phospholipid were found to penetrate extensively into the d-OTS layer with the mixed chain region (d-OTS and DMPC) having a total thickness of 30.5 Å. This mixed region was divided into two halves for analysis; the 'lower half' (nearest to the substrate surface) was found to comprise anchored d-OTS chains mixed with the lipid chains in the volume ratio approx. 0.60:0.35. The corresponding volume ratio in the 'upper half' of this region was determined to be approx. 0.50:0.40. The thicknesses of these regions were found to be 17.9 Å (incorporating approx. 6% solvent) and 12.6 Å (incorporating approx. 9% solvent) for the lower and upper halves respectively. The DMPC head groups were found to be confined to the most external layer (furthest away from the silicon substrate). This layer was found to have a thickness of 9.4 Å and included a small fraction of the lipid alkyl chains with approx. 47% solvent. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Phospholipid; Dimyristoylphosphatidylcholine; Neutron reflection; Langmuir-Blodgett; Surfactant monolayer

### 1. Introduction

There is currently considerable interest in the application of biomembrane lipids as stationary phases

in high-performance liquid chromatography (HPLC) as models for the prediction of drug partitioning into lipid liposomes and biological membranes [1–3]. These chromatographic systems are based on either covalent bonding, via one of the phospholipid alkyl chains to the stationary phase (the so-called immobilised artificial membrane or IAM phases) [1–3] or alternatively dynamic coating of silica [4] or reversed phase [5] HPLC stationary phases with phospholipid. We have previously reported on the preparation and characterisation of dynamically coated reversed

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phase HPLC (RP-HPLC) columns using a variety of biomembrane lipids [5,6] and in order to establish a phase structure based interpretation of the chromatographic data obtained we have undertaken a systematic study of the structure of supported phospholipid monolayers. In this report, we present the first of our findings, giving the structure of a supported monolayer of L- $\alpha$ -dimyristoylphosphatidylcholine (DMPC), determined using the technique of neutron specular reflection [7–9]. The DMPC monolayer was adsorbed onto a silicon block which had been previously hydrophobised by bonding with deuterated octadecyltrichlorosilane (d-OTS). Previous experiments by other groups have determined the structure of bilayers of DMPC deposited onto (hydrophilic) silicon using the Langmuir-Blodgett technique [10] and onto (hydrophilic) quartz by the spontaneous collapse of vesicles [11]. In addition, Charitat and co-workers have recently investigated the structure of dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) bilayers at the solid-liquid interface deposited onto (hydrophilic) silicon using a combination of Langmuir-Blodgett and Langmuir-Schaefer techniques [12]. This present study, however, is the first to determine the structure of a *monolayer* of DMPC by neutron reflection at the solid-liquid interface deposited onto a *hydrophobic* silicon support using the Langmuir-Blodgett technique.

## 2. Theory of neutron reflection

In a neutron reflection experiment, the elastic scattering (specular reflection),  $R$ , is determined as a function of the wave vector (momentum) transfer,  $\kappa$ , perpendicular to the reflecting surface, where:

$$\kappa = \frac{4\pi \sin \theta}{\lambda} \quad (1)$$

$\lambda$  is the wavelength of the incident neutron beam and  $\theta$  its grazing angle of incidence.  $R(\kappa)$  is related to the scattering length density across the interface,  $\rho(z)$  by:

$$R(\kappa) = \frac{16\pi^2}{\kappa^2} |\rho(\kappa)|^2 \quad (2)$$

where  $|\rho(\kappa)|$  is the one-dimensional Fourier trans-

form of  $\rho(z)$ :

$$\rho(\kappa) = \int_{-\infty}^{+\infty} \exp(-i \kappa z) \rho(z) dz \quad (3)$$

and  $\rho(z)$  is a function of the distance perpendicular to the interface.

These equations apply in the kinematic region, well away from the critical edge and for very thin films.

Although the structures of both the monolayer and substrate are directly related to their reflectivity, in practise, data analysis is often performed using the optical matrix method [7,9,13–15]. In this method, the experimentally derived data are compared with the profile calculated from a structural model. The model usually consists of a series of layers each with its own thickness,  $\tau$ , and scattering length density,  $\rho$ . Additionally, a value for the interfacial roughness,  $\sigma$ , of each layer may be included in the calculations, if considered necessary. Variation of  $\tau$ ,  $\rho$  and  $\sigma$  for each layer, followed by subsequent comparisons of the experimental and calculated reflectivity profiles can then be carried out until the best fit is obtained. Although good agreement between these calculated and experimental profiles indicates that the proposed model may be accurate, it does not prove that the ‘correct answer’ has been found since more than one combination of scattering length density profiles may give the same reflectivity profile [16,17]. This leads to the rationale for the use of isotopic substitutions and contrast variation which rely on the principle that different nuclei scatter neutrons differently and, in the case of protons and deuterons, this scattering is of the opposite phase. These techniques thus provide the means to highlight particular parts of the system under study by simply altering the H:2H ratios. Of particular importance is the fact that solvents may be prepared to match the scattering length density of the substrate (in this case silicon) so that there is no contrast between that and the solvent. The arising reflectivity profile is then a function only of the interfacial material. Whilst this method of data analysis has its limitations [18] it is the best available technique for determining the structure of interfacial layers at the solid-liquid interface.

Silicon is commonly used as the substrate for neutron reflection experiments for the following reasons [19]. Firstly, it is available in large sizes as a single

crystal; secondly, it is transparent to neutrons which means the beam can be passed through it to the interface under study; and finally, its surface may be modified to produce many different functionalities.

### 3. Experimental section

The substrate used in these investigations was a single crystal of silicon of approximate size 12.5 cm × 5 cm × 2.5 cm. One large surface (111) of this block had been previously polished and rendered hydrophobic by derivatisation with octadecyltrichlorosilane in its deuterated form ( $C_{18}D_{37}SiCl_3$ , d-OTS). The procedures for cleaning the substrate and derivatisation of it with d-OTS have been described in detail elsewhere [20,21].

10 mg DMPC (Sigma, UK) was dissolved in 5 ml chloroform to produce a 2 mg/ml solution. 60  $\mu$ l of this solution was then added dropwise to the water (18.2 M $\Omega$  purity, Elga, UK) surface of a large (approx. 10 dm<sup>3</sup> volume, approx. 0.14 m<sup>2</sup> surface area) Langmuir trough (Joyce Loebel, UK) using a Hamilton microsyringe (Aldrich, UK). Evaporation of the solvent and subsequent formation of the DMPC monolayer was allowed to occur over a 20 min period after which time the DMPC monolayer was compressed and maintained at a surface pressure of 40 mN/m (before its collapse point) throughout the dipping procedure. The block (to which d-OTS was already bonded) was then passed down through the air-water interface, with its large, measurable face vertical, at a speed of 4 mm/min using the conventional Langmuir-Blodgett technique [22]. This procedure resulted in a monolayer of DMPC being deposited onto the block with the hydrophilic head group region oriented away from the surface. The temperature was maintained at 20 ± 1°C during the Langmuir-Blodgett deposition procedure and as such the DMPC exists in the gel phase throughout. The transfer ratio (the reduction in water surface area occupied by the lipid molecules divided by the total surface area of the silicon block) was calculated as close to unity and as such the deposition was deemed successful. The surface pressure was continuously measured throughout the procedure using a Wilhelmy plate made from 3 cm × 1 cm filter paper (Whatman,

UK) attached to a microbalance (C.I. Electronics, UK). With the coated substrate still submerged, excess DMPC on the water surface was siphoned off using a Pasteur pipette (John Poulten, UK) attached to a vacuum pump (Tokyo Rikakikai, Japan). The lipid-coated block was then withdrawn from the water at a speed of 13.5 mm/min, leaving the DMPC monolayer attached to the hydrophobic substrate with the head groups exposed. After withdrawal of the block, the barrier was closed to the minimum surface area possible in order to determine if any lipid had been transferred back onto the water surface. A surface pressure increase of 1–2 mN/m was obtained, indicating that the withdrawal procedure did not result in significant loss of lipid from the block. The block was then left to dry for 1 h in a laminar flow hood.

After the neutron specular reflection experiments had been performed, the DMPC was removed from the surface of the block by repeated rinsing with methanol followed by 60 min sonication in a mixture of chloroform and methanol (1:1 v/v) using a bath sonicator (FS minor, Decon Ultrasonics, UK). This process was followed by further rinsing of the block with methanol to remove all traces of the lipid whilst leaving the d-OTS layer intact. All solvents used were of HPLC-grade quality, supplied by Rathburn (UK).

Neutron reflectivity measurements were performed (using a pulsed white beam neutron source) on the CRISP reflectometer [7,23] at the Rutherford Appleton Laboratory (RAL) (Didcot, UK). The silicon block was clamped into a solid-liquid cell, trapping a thin layer of solvent between its measurable face and a shallow Teflon trough. The structure and dimensions of this apparatus have been described in detail elsewhere [8,20]. The assembled cell was aligned in the neutron reflectometer with the block's highly polished (measurable) face pointing down so that the beam passed through the sample before being reflected up into the detector. Measurements were made (at room temperature) at incident neutron beam angles of 0.35°, 0.8° and 1.8° to cover a momentum transfer range of 0.012–0.5 Å<sup>-1</sup>.

The d-OTS-bonded block, i.e. without the DMPC monolayer attached, was first characterised by measuring the neutron reflectivity with the block immersed in a subphase of 6:4 v/v <sup>2</sup>H<sub>2</sub>O:H<sub>2</sub>O which gives a contrast match to silicon (abbreviated as

Table 1  
Properties of materials used in this study

Material	Volume <sup>a</sup> (Å <sup>3</sup> )	Scattering length, $b^b$ ( $\times 10^4$ Å)	Scattering length density, $\rho$ ( $\times 10^6$ Å <sup>-2</sup> )
Si	20	0.42	2.07
SiO <sub>2</sub>	47	1.59	3.41
H <sub>2</sub> O	30	-0.17	-0.56
D <sub>2</sub> O	30	1.91	6.35
CmSi	30	0.62	2.07
-C <sub>18</sub> D <sub>37</sub> (d-OTS)	542	36.65	6.76
DMPC	1024	3.10	0.28
DMPC head group	268	5.98	1.20
DMPC alkyl chains	756	-2.91	-0.49

<sup>a</sup>Volumes calculated from density data.

<sup>b</sup>[34].

cmSi). Subsequently, the lipid coated block was characterised using three different contrasts, employing the subphases <sup>2</sup>H<sub>2</sub>O, H<sub>2</sub>O and cmSi. Absolute scaling of the reflectivity data was achieved with reference to the critical edge for the d-OTS-bonded block immersed in a subphase of <sup>2</sup>H<sub>2</sub>O. All reflectivity profiles were modelled according to optical matrix methods [7,9,13–15] using the molecular volumes, atomic scattering lengths and scattering length densities given in Table 1. The final fits between the calculated and experimental data profiles were optimised by least squares refinement [9,14,25] of the layer thicknesses, scattering length densities and sample backgrounds.

In order to determine the molecular composition of each layer within the modelled multilayers, the fitted values of the scattering length densities of the layers were taken for each of the different contrast runs and multiple regression used to solve the set of equations:

$$\rho_{\text{layer}} = \sum f_i \rho_i + (1 - \sum f_i) \rho_s \quad (4)$$

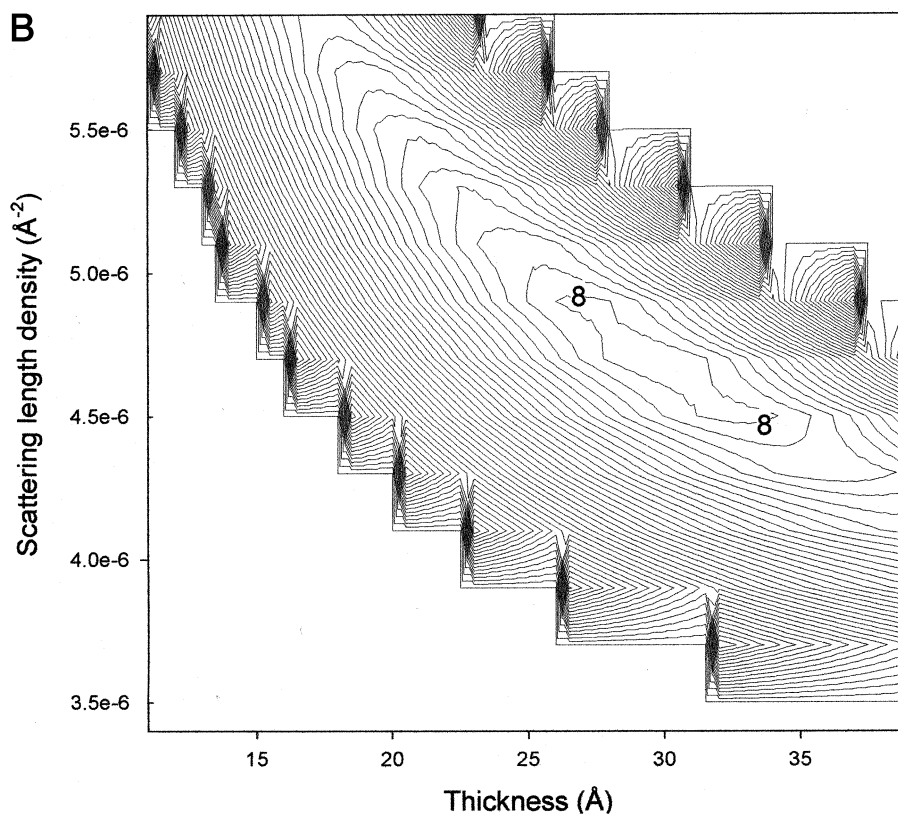
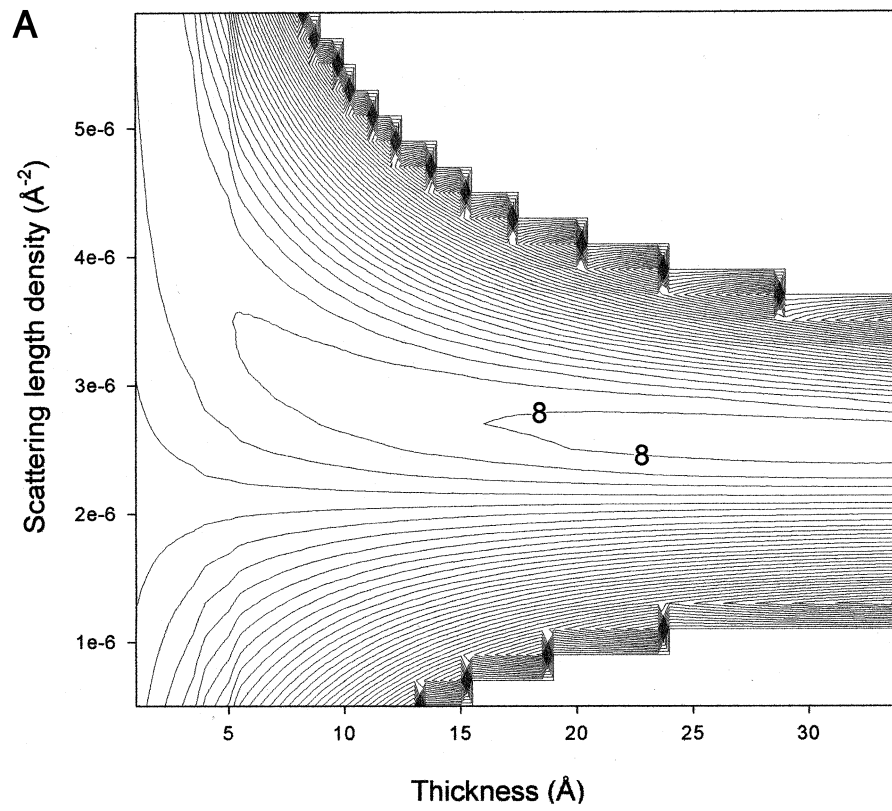
where  $\rho_{\text{layer}}$  are the fitted values of the scattering length densities of the given layer,  $\rho_s$  are the scattering length densities of the various solvents employed, and  $f_i$  and  $\rho_i$  are the volume fractions and scattering length densities of the other layer components (i.e. SiO<sub>2</sub> and/or d-OTS and/or DMPC).

#### 4. Results and discussion

In investigations of the structure of multilayers at the solid-liquid interface, it is imperative that optimum characterisation of the bare substrate surface is achieved as a means to aid subsequent investigations after the deposition of additional layers. It is important to note at the outset, therefore, that it is not ideal to characterise the structure of the d-OTS-bonded block using a single reflectivity profile (in this case cmSi). It would have been preferable to carry out experiments to characterise the oxide layer on the silicon block prior to its derivatisation with d-OTS, and likewise to follow the approach taken by others [12,16,20,23] in using several solvent contrasts to better define the structure of the bonded d-OTS layer. Owing to various practical limitations, however, we were unable to make these additional measurements. Previous experiments [20], however, have indicated that there is no need to characterise the bare oxide layer prior to bonding with d-OTS because the derivatisation process significantly changes its composition.

As an alternative to characterising the d-OTS-bonded block in additional solvent contrasts, we elected to follow a Bayesian approach in modelling the reflectivity data [28] and systematically explored the problem landscape to find the solution of max-

Fig. 1. Variation in  $\chi^2$  as a function of the thickness and scattering length densities of the silicon oxide layer (A) and d-OTS layer (B) of the hydrophobised silicon block. The values of  $\chi^2$  (here shown contoured at intervals of 4) quantify the extent of fit between the observed and calculated reflectivity data, with the calculated reflectivity determined for set values of the thicknesses and scattering length densities of the layers. The lowest contour level in the plots (for  $\chi^2 = 8$ ) are labelled.



imum likelihood. We assumed a simple two-layer model for the d-OTS-bonded block, and then compared the calculated [3,9–11] and observed reflectivity profiles with the thicknesses of the two layers sampled at 0.5 Å intervals in the range 0.5–45 Å, and their scattering length densities sampled at intervals of  $0.2 \times 10^{-6} \text{ Å}^{-2}$  in the range  $0.4 \times 10^{-6}$ – $6.4 \times 10^{-6} \text{ Å}^{-2}$ . Throughout this search, the scattering length density of the solvent was fixed at  $2.1 \times 10^{-6} \text{ Å}^{-2}$  and the level of background scattering fixed at  $2 \times 10^{-6} \text{ Å}^{-2}$ . For each model tested the fit between the observed and calculated reflectivity data ( $R_k^o$  and  $R_k^c$ , respectively) was quantified as:

$$\chi^2 = \Sigma (R_k^o - R_k^c) / \sigma_k^2 \quad (5)$$

where  $\sigma_k$  are the errors on  $R_k^o$ . In preference to simply minimising  $\chi^2$  by iterative least squares refinement of the thicknesses and scattering length densities of the two layers, we elected first to visualise the probability density function, taking a series of two-dimensional sections through the four-dimensional parameter space and searching for minima in  $\chi^2$ . Fig. 1 shows two of these sections.

Fig. 1A shows the variation in  $\chi^2$  as a function of the thickness ( $\tau_1$ ) and scattering length density ( $\rho_1$ ) of the silicon oxide layer (with the d-OTS layer thickness,  $\tau_2$ , and scattering length density,  $\rho_2$ , fixed at the arbitrary but physically reasonable values of 33.5 Å and  $4.5 \times 10^{-6} \text{ Å}^{-2}$ , respectively). It will be noted that there are numerous steep-sided minima in the  $\chi^2$  function at both the upper and lower boundaries of the terrain and a more broad minimum centred around  $\rho_1 = 2.8 \times 10^{-6} \text{ Å}^{-2}$ . While the former all turn out to be local minima with relatively high  $\chi^2$ , the latter is much deeper and is presumed to encompass the global minimum. Likewise, in Fig. 1B, where we show the variation in  $\chi^2$  as a function of  $\tau_2$  and  $\rho_2$  (with  $\tau_1$  and  $\rho_1$  fixed at 22.6 Å and  $2.75 \times 10^{-6} \text{ Å}^{-2}$  respectively), we again see numerous shallow local minima at the boundaries of the terrain, and a deeper and more broad minimum in the region of  $\tau_2 = 26$ – $34 \text{ Å}$ ,  $\rho_2 \approx 4.6 \times 10^{-6} \text{ Å}^{-2}$ . Given the confidence afforded by these systematic analyses of all of the *physically acceptable* models of the d-OTS-bonded silicon block, we proceeded to use a least squares refinement procedure to arrive at our final solution, with the starting point for the minimisation taken as

the focal point of the (presumed) global minimum identified from the  $\chi^2$ -contour plots.

The calculated and observed reflectivity profiles are shown in Fig. 2A and the corresponding least squares refined layer thicknesses and scattering length densities obtained were, respectively, 22.6 Å and  $2.75 \times 10^{-6} \text{ Å}^{-2}$  for layer 1, and 33.5 Å and  $4.5 \times 10^{-6} \text{ Å}^{-2}$  for layer 2.

The silicon substrate was determined to have an interfacial roughness value of approx. 4 Å, which is generally considered as a smooth surface [26]. The first layer (nearest to the silicon block) was found to be composed of 50% silicon oxide with the remaining 50% of the volume occupied by solvent molecules. The second layer was found to consist of 52% d-OTS and 48% solvent. This gave the mean area per molecule of d-OTS as  $31 \text{ Å}^2$  with the level of hydration approx. 17 solvent molecules per d-OTS chain. The coverage of d-OTS on the block was thus found to be 68%, similar to that found by Penfold and co-workers [30]. (Note, however, that the percentage composition values calculated and quoted here must be regarded as approximate, given that they are derived from just one set of reflectivity data collected using a single solvent contrast.)

In the present investigation, the thickness of d-OTS was calculated as 33.5 Å (layer 2, Table 2). Other groups, however, have found the thickness of d-OTS to be 24 Å [16] and 28 Å [24] using the same technique of neutron reflection at the solid-liquid interface. These investigators divided their d-OTS-bonded blocks into a three-layer model for analysis – with the extra layer required to fit the reflectivity profile for the d-OTS-bonded block in  $^2\text{H}_2\text{O}$ . In these analyses, layer 1 consisted of silicon oxide (as in this study), layer 2 was deemed to be a ‘crystalline’ form of d-OTS with layer 3 also comprising d-OTS, but in a more liquid-like state due to extra solvent

Table 2  
Model-fitted parameters for DMPC/d-OTS block

Layer	Thickness (Å)	$\rho$ ( $\times 10^6 \text{ Å}^{-2}$ )		
		$^2\text{H}_2\text{O}$	$\text{H}_2\text{O}$	cmSi
1	22.6	2.936	2.642	2.803
2	17.9	4.148	3.975	4.399
3	12.6	4.161	3.499	3.963
4	9.4	3.136	−0.060	1.140

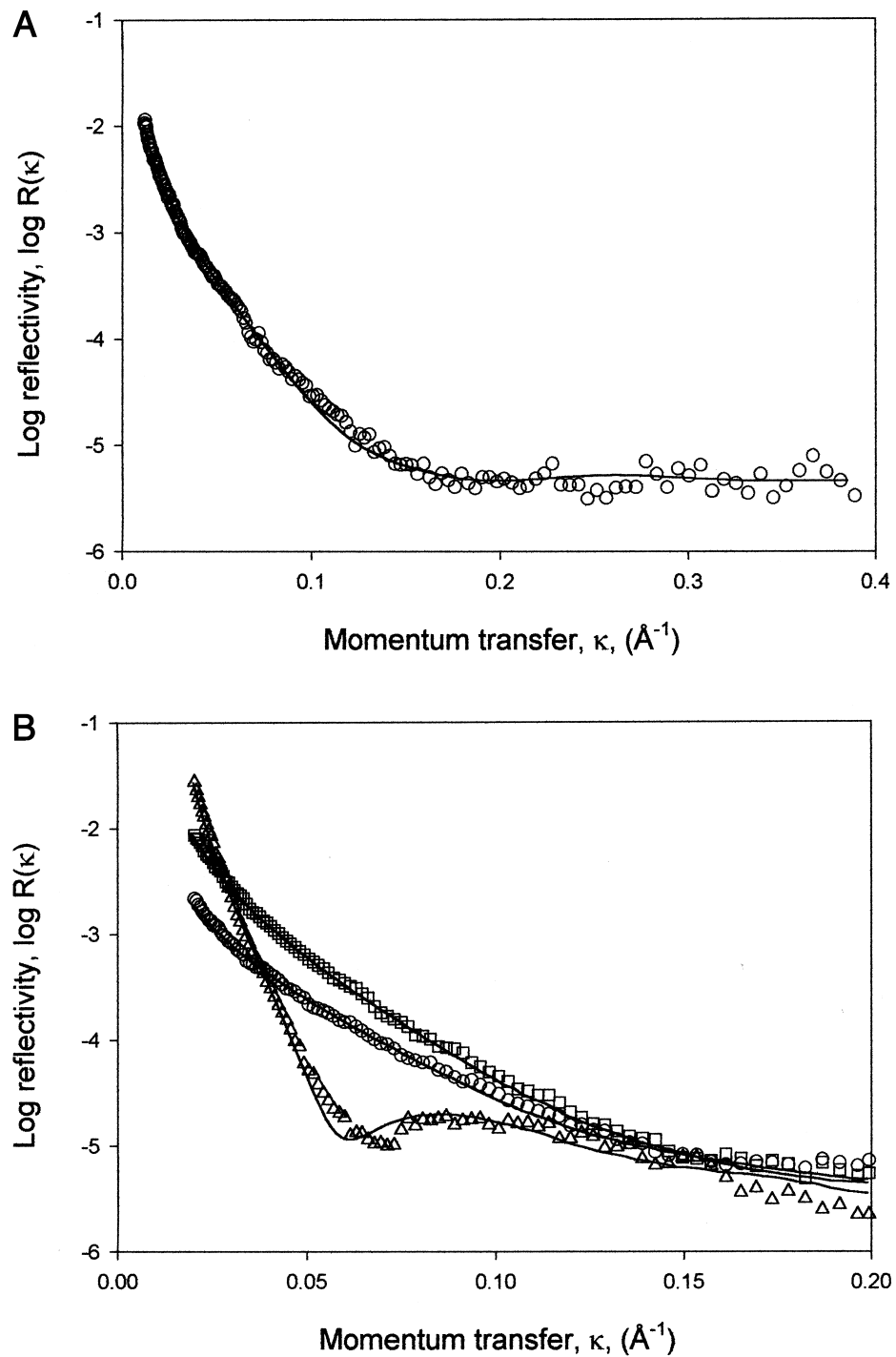


Fig. 2. Reflectivity profiles and fitted curves for the uncoated hydrophobised silicon block in water contrast-matched to silicon (cmSi) (A) and for the same block coated with DMPC in the three different solvents:  $^2\text{H}_2\text{O}$  ( $\Delta$ ),  $\text{H}_2\text{O}$  ( $\square$ ) and cmSi ( $\circ$ ) (B). The symbols represent the experimental data and the continuous lines show the model-fitted profiles calculated according to optical matrix methods using the parameters detailed in Tables 1 and 2.

penetration. The area per molecule of d-OTS calculated by these groups is broadly consistent with the present study (for example, Fragneto and co-workers [24] calculated the ‘liquid-like’ region of d-OTS to have an area per molecule of  $28 \pm 1 \text{ \AA}^2$ ). It should be noted, however, that in the present work there was significantly more solvent found to be associated with the d-OTS chains, presumably as a result of the 68% coverage of d-OTS on the silicon block.

With the structure of the d-OTS-bonded block so determined, the three sets of reflectivity data obtained following coating of the block with DMPC were fitted simultaneously assuming various different multilayer models. The fits obtained assuming both two- and three-layer models were rather poor, and the fit for a four-layer model perfectly acceptable. The fitted profiles obtained using this four-layer model are shown in Fig. 2B and the corresponding least squares refined layer thicknesses and scattering length densities are shown in Table 2.

Layer 1 (closest to the silicon block) was again assumed to consist of silicon oxide, layers 2 and 3 of d-OTS and DMPC, and layer 4 of DMPC only. Each layer was also assumed to contain some quantity of solvent. The thickness of the silicon oxide, layer 1, was constrained in this analysis to the value determined for the d-OTS-bonded block ( $22.6 \text{ \AA}$ ).

The composition of the layers (determined using Eq. 4) was found to be: layer 1,  $90 \pm 13\%$  silicon oxide with solvent molecules occupying the remaining volume; layer 2,  $59 \pm 2\%$  d-OTS,  $35 \pm 3\%$  DMPC and  $6\%$  solvent (calculated by difference) with a hydration of approx. one solvent molecule per DMPC molecule; layer 3,  $52 \pm 2\%$  d-OTS,  $39 \pm 3\%$  DMPC and  $9\%$  solvent (calculated by difference) with a hydration of approx. 2.3 solvent molecules per DMPC molecule; and finally, layer 4,  $53 \pm 1\%$  DMPC. This layer was assumed to involve all of the phospholipid head groups and some of the lipid alkyl chains, with solvent occupying the remaining 47% of the space, giving a hydration of approx. 8.9 solvent molecules per DMPC. These composition data indicate that around 0.3 of the DMPC is located in layer 4, 0.3 in layer 3 and the remaining 0.4 in layer 2. Around 54% of the DMPC alkyl chains (which amounts to the two terminal methyl groups and approx. 12 methylene groups) are thus found in layer 2. A further 42% of the alkyl chains (amounting to approx.

11 methylene groups) are located in layer 3. Layer 4 involves all of the DMPC phospholipid head group region and approx. one alkyl chain methylene group. The mean area per molecule of DMPC was estimated to be  $60.5 \text{ \AA}^2$ . A schematic diagram showing the modelled structure of the interfacial multilayer is presented in Fig. 3.

It must be emphasised here, however, that although this (four-layer) model for the supported d-OTS/DMPC layer appears physically reasonable, and although it gives a statistically acceptable description of the measured reflectivity data, it cannot (as was noted earlier) be regarded as a unique solution. There will undoubtedly be other molecular models that would yield the same neutron reflectivity profiles and which would also be just as tenable on structural grounds. Nevertheless, it must be stressed that it is highly unlikely that the estimate made for the combined overall thickness of the adsorbed layer is much in error and given that the measured thickness is far less than the sum of the extended chain lengths for d-OTS and DMPC (see below) we can be confident in concluding that this system is best described as a mixed monolayer rather than a ‘true’ bilayer. Now, whether or not the d-OTS and DMPC alkyl chains are as regularly interdigitated as indicated in Fig. 3A is much more debatable. We have no way of knowing, for example, whether the 68% coverage of the silicon block by d-OTS represents an even but relatively sparse covering of the entire silicon surface by d-OTS chains, or whether it represents a complete and compact coverage of approx. two-thirds of the surface with the remaining one-third comprising islands of bare solid with no grafted  $C_{18}$  chains. In the arguments presented above we have indirectly assumed that it is the former situation which prevails, but in the event that it is the latter, then the modelled interpenetration of the DMPC and d-OTS chains will actually be more apparent than real. In other words, the DMPC molecules in this system might be clustered to ‘fill in’ the various voids in the forest of octadecyl chains (as shown in Fig. 3B). On statistical grounds, therefore, it is probably safest to assume that the supported d-OTS/DMPC adsorbed layer involves a mixture of both types of arrangement.

Experiments have previously been performed with phospholipids, single, double and branched-chained



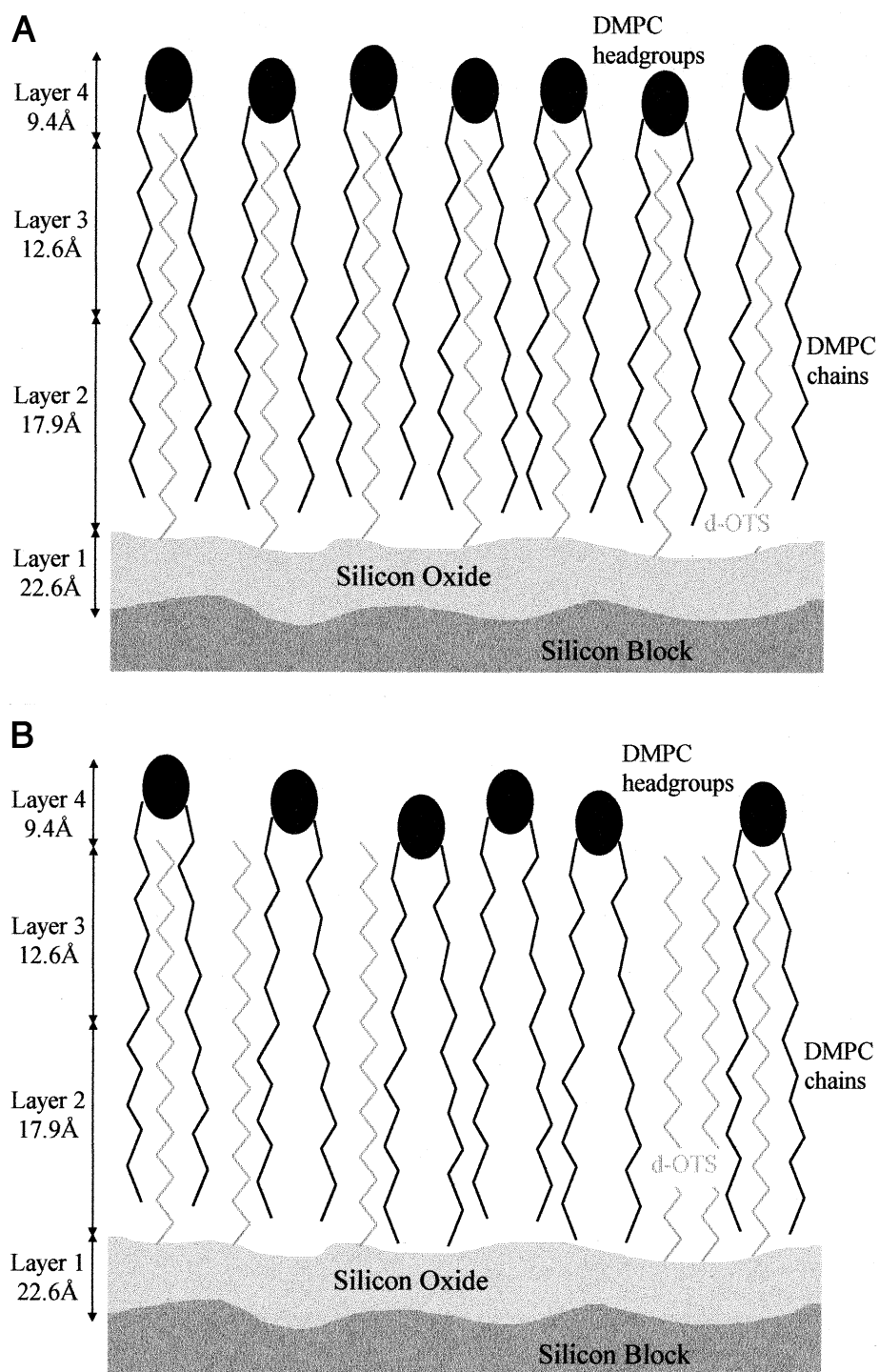


Fig. 3. A cartoon representation of the molecular structure of the DMPC-coated hydrophobised silicon surface. In A the d-OTS chains are shown to be evenly but sparsely arrayed on the silicon surface, with the DMPC molecules fairly regularly spaced with their alkyl chains interdigitating with those of the d-OTS. In B, the d-OTS chains are shown to be more compactly arrayed over part of the silicon surface but with other areas of the silicon entirely devoid of d-OTS. Here, the DMPC molecules are shown in-filling the voids in the d-OTS layer.

surfactants using the surface force apparatus [31] which have also shown that interdigitation can occur when the alkyl chain regions of two monolayers are brought into contact. This same general system structure has been elucidated by other groups (also using neutron reflection) [13,26] who also found lipid alkyl chains penetrated into a hydrophobic d-OTS region.

The values obtained for the thicknesses of each of the layers (Table 2) correspond well with data previously obtained by other groups studying similar systems. Layer 1 was found to be 22.6 Å thick, in keeping with the silicon oxide layer thicknesses calculated by other groups using the same technique [16,24,27]. The combined thicknesses of layers 2 and 3 (both consisting of d-OTS and lipid alkyl chains but with varying amounts of solvent) was found to be 30.5 Å. This value is slightly higher than the d-OTS layer thickness (28 Å) reported by Fragneto and co-workers [24], presumably due to incomplete penetration of the DMPC into the d-OTS layer in the present study. As such, a small part of layer 3 (approx. 2.5 Å) would solely consist of DMPC alkyl chains and solvent. Layer 4 was assumed to consist only of DMPC head groups and solvent and was found to have a thickness of 9.4 Å, a value in agreement with those obtained by other workers who found the phosphatidylcholine head group length to be  $8 \pm 1.5$  Å in bilayers of DPPC and DSPC on a silicon substrate [12,32] and bilayers of DMPC on a quartz substrate [11]. The slight difference between the results from the present study and those previously reported [11,12,32] may be attributed to the use of a hydrophobic substrate supporting the phospholipid monolayer rather than a hydrophilic substrate supporting a phospholipid bilayer [10–12].

Finally, as was noted above, the total combined thicknesses of the d-OTS and DMPC-containing layers is significantly less than the sum of the octadecyl chain and phospholipid extended lengths (approx. 40 Å as opposed to approx. 24 Å plus approx. 25 Å = 49 Å). In their work on mixtures of sodium dodecyl sulphate and dodecyl betaine, Hines and co-workers [16] made the same observation and attributed this to a tilting of the lipid chains. Here, however, we attribute the reduction in the adsorbed layer thickness to an *interdigitation* of the d-OTS and

DMPC alkyl chains. We believe that this arises primarily as a consequence of the rather low (68%) coverage of the d-OTS layer. It may also be, however, that there is a contribution from the fact that, at the temperature of these experiments (which was maintained as best as could be managed at around 20°C), the DMPC exists in the gel rather than the fluid state since differential scanning calorimetry (DSC) and deuterium nuclear magnetic resonance ( $^2\text{H-NMR}$ ) experiments performed by Käsbauer and Bayerl [33] have already indicated an interdigitation of  $\text{C}_{18}$  chains and lecithin monolayers under gel phase conditions.

In conclusion, the structure of a DMPC monolayer adsorbed onto a silicon surface, previously hydrophobised by bonding with d-OTS, has been characterised. Rather than forming the 'traditional' bilayer structure, significant interdigitation between the bonded d-OTS and alkyl chains of the coated phospholipid was observed. Assuming that the above system serves as an appropriate model for a phospholipid coated RP-HPLC phase then such columns should provide similar surfaces to the IAM phases. Essentially these phases resemble half a membrane lipid bilayer with the polar head groups protruding away from the silica surface providing the initial analyte-phase interaction site. Thus, the coated phases should yield chromatographic data comparable to those obtained using the covalently bonded IAM phases.

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### References

- [1] S. Ong, H. Liu, X. Qui, B.G., C. Pidgeon, *Anal. Chem.* 67 (1995) 755.
- [2] C. Pidgeon, S. Ong, H. Liu, X. Qui, M. Pidgeon, A.H. Dantzig, J. Munroe, W.J. Hornback, J.S. Kashner, L. Glunz, T. Szczerba, *J. Med. Chem.* 38 (1995) 590.

- [3] C.Y. Yang, S.J. Cai, H.L. Lui, C. Pidgeon, *Adv. Drug Deliv. Rev.* 23 (1996) 229.
- [4] K. Miyake, F. Kitaura, N. Mizuno, H. Terada, *J. Chromatogr.* 389 (1987) 47.
- [5] M. Hanna, V. de Biasi, B. Bond, C. Salter, A.J. Hutt, P. Camilleri, *Anal. Chem.* 70 (1998) 2092.
- [6] M. Hanna, V. de Biasi, B. Bond, P. Camilleri, A.J. Hutt, *J. Pharm. Pharmacol.* 51 (Suppl.) (1999) 215.
- [7] D.G. Bucknall, Neutron reflection studies of polymers, in: R.A. Pethrick, J.V. Dawkins (Eds.), *Modern Techniques for Polymer Characterisation*, John Wiley and Sons, Chichester, 1999, p. 109.
- [8] J.S. Higgins, H.C. Benoît, *Polymers and Neutron Scattering*, Clarendon Press, Oxford, 1996.
- [9] J. Penfold, R.K. Thomas, *J. Phys. Condens. Mat.* 2 (1990) 1369.
- [10] L.K. Tamm, H.M. McConnell, *Biophys. J.* 47 (1985) 105.
- [11] S.J. Johnson, T.M. Bayerl, D.C. McDermott, G.W. Adam, A.R. Rennie, R.K. Thomas, E. Sackmann, *Biophys. J.* 59 (1991) 289.
- [12] T. Charitat, E. Bellet-Amalric, G. Fragneto, F. Graner, *Eur. Phys. J. B* 8 (1999) 583.
- [13] J.R. Lu, R.K. Thomas, *J. Chem. Soc. Faraday Trans.* 94 (1998) 995.
- [14] J.R. Lu, E.M. Lee, R.K. Thomas, *Acta Crystallogr. A* 52 (1996) 11.
- [15] M. Born, E. Wolf, *Principles of Optics*, Pergamon Press, Oxford, 1983.
- [16] J.D. Hines, G. Fragneto, R.K. Thomas, P.R. Garrett, G.K. Rennie, A.R. Rennie, *J. Coll. Int. Sci.* 189 (1997) 259.
- [17] E.M. Lee, J.E. Milnes, *J. Appl. Crystallogr.* 28 (1995) 518.
- [18] M. Schalke, P. Krüger, M. Weygand, M. Lösche, *Biochim. Biophys. Acta* 1464 (2000) 113.
- [19] Z.X. Li, P.N. Thirtle, A. Weller, R.K. Thomas, J. Penfold, J.R.P. Webster, A.R. Rennie, *Physica B* 248 (1998) 171.
- [20] G. Fragneto, J.R. Lu, D.C. McDermott, R.K. Thomas, A.R. Rennie, P.D. Gallagher, S.K. Satija, *Langmuir* 12 (1996) 477.
- [21] T. Vallant, H. Brunner, U. Mayer, H. Hoffmann, T. Leitner, R. Resch, G. Friedbacher, *J. Phys. Chem. B* 102 (1998) 7190.
- [22] G. Roberts, *Langmuir-Blodgett Films*, Plenum Press, New York, 1990.
- [23] J. Penfold, R.C. Ward, W.G. Williams, *J. Phys. E Sci. Instrum.* 20 (1987) 1411.
- [24] G. Fragneto, Z.X. Li, R.K. Thomas, A.R. Rennie, J. Penfold, *J. Coll. Int. Sci.* 178 (1996) 531.
- [25] T.L. Crowley, E.M. Lee, E.A. Simister, R.K. Thomas, *Physica B* 173 (1991) 143.
- [26] G. Fragneto, R.K. Thomas, A.R. Rennie, J. Penfold, *Langmuir* 12 (1996) 6036.
- [27] P.N. Thirtle, Z.X. Li, R.K. Thomas, A.R. Rennie, S.K. Satija, L.P. Sung, *Langmuir* 13 (1997) 5451.
- [28] D.S. Sivia, J.R.P. Webster, *Physica B* 248 (1998) 327.
- [30] J. Penfold, R.M. Richardson, A. Zarbakhsh, J.R.P. Webster, D.G. Bucknall, A.R. Rennie, R.A.L. Jones, T. Cosgrove, R.K. Thomas, J.S. Higgins, P.D.I. Fletcher, E. Dickinson, S.J. Roser, I.A. McLure, A.R. Hillman, R.W. Richards, E.J. Staples, A.N. Burgess, E.A. Simister, J.W. White, *J. Chem. Soc. Faraday Trans.* 93 (1997) 3899.
- [31] Y.L. Chen, C.A. Helm, J.N. Israelachvili, *J. Phys. Chem.* 95 (1991) 10736.
- [32] H. Reinl, T. Brumm, T.M. Bayerl, *Biophys. J.* 61 (1992) 1025.
- [33] M. Käsbauer, T.M. Bayerl, *Langmuir* 15 (1999) 2431.
- [34] V.F. Sears, *Neutron News* 3 (1992) 26.