

## Structure of bidimensional phospholipidic crystallites on formamide determined by X-ray diffraction

Arnaud Saint-Jalmes <sup>a</sup>, François Graner <sup>a,1</sup>, François Gallet <sup>a</sup>, Pierre Nassoy <sup>b</sup>, Michel Goldman <sup>b,2</sup>

<sup>a</sup> *Laboratoire de Physique Statistique de l'Ecole Normale Supérieure (URA 1306 associée au CNRS et aux Universités Paris VI et Paris VII), 24, rue Lhomond, 75231 Paris Cedex 05, France*

<sup>b</sup> *Laboratoire de Physique des Surfaces et Interfaces, Institut Curie, 11, rue Pierre et Marie Curie, 75231 Paris Cedex 05, France*

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### Abstract

By using grazing incidence X-ray diffraction, we determined the structure of the solid phase of a C<sub>18</sub>–C<sub>18</sub> phosphocholine monolayer spread at the air–formamide interface. The chains are organized on an oblique lattice, and their tilt, measured from Bragg rod scans, is about 35°. No structural change is observed when the surface pressure is varied from 1 to 38 mN/m. In the solid phase, the area per molecule is 46 Å<sup>2</sup>, and the compression modulus is large. The phase stiffness is dominated by the interactions between the polar heads and the formamide, more than between the aliphatic chains.

### 1. Introduction

Amongst other liquids, formamide can be used as an alternative non-aqueous solvent to support amphiphilic monolayers [1–3]. The aim is to understand the specific interactions between polar heads, and with the substrate, by comparing the film behaviour on different liquids. Recently, the phase diagram of 1,2-distearoyl-sn-glycero-3-phosphatidylcholine (DSPC) on formamide has been determined from surface isotherms and fluorescence microscopy observations [4]. One recovers the same variety of

phases as on water (gas, liquid, solid and mesophases), but not at the same relative position [5]. At this stage, the phase diagram must be completed by diffraction experiments. For example, X-ray scattering experiments have shown that, for phosphatidylethanolamines on water, there is a transition between a tilted and a non-tilted solid phase as the surface pressure increases [6]. No such evidence could be found from DSPC isotherms on formamide. Moreover, a specific feature occurring on formamide is the spontaneous and stable buckling of the low temperature solid phase, above a pressure threshold  $\Pi_c \approx 7$  mN/m [7]<sup>3</sup>. This phenomenon has never

<sup>1</sup> Now at Laboratoire de Spectrométrie Physique, BP 87, 38402 St Martin d'Hères, France.

<sup>2</sup> Also at LURE, Bâtiment 209 D, Université Paris Sud, 91405 Orsay Cedex, France.

<sup>3</sup> Since this work, the pressure threshold for stable permanent buckling has been found lower, at 7 mN/m.

been observed on water. Looking for a possible tilted/untilted transition in the solid phase, which could maybe induce buckling, was also a motivation for this work.

In this Letter, after a brief description of the experimental apparatus and procedure, we present in situ X-ray diffraction data for the DSPC solid phase at  $T = 10^\circ\text{C}$ . The diffracted intensity presents three narrow peaks, corresponding to an oblique lattice of chains. The area per molecule is  $46 \text{ \AA}^2$ . Out-of-plane analysis (Bragg rod scans) shows that the chain tilt is  $35^\circ$  and the monolayer thickness is about  $25 \text{ \AA}$ . The compression modulus  $K = -A(d\Pi/dA) = 3500 \text{ mN/m}$  of the solid phase is large, and no structural change is detected in the accessible surface pressure range ( $1 < \Pi < 38 \text{ mN/m}$ ). The results are compared to available data concerning phospholipids on water.

## 2. Experiments

Grazing incidence X-ray diffraction was carried out using the D24 beam line of DCI, in LURE, Orsay (France). A general description of the beam set-up and Langmuir trough can be found elsewhere [8]. The beam is nearly horizontal, its wavelength  $\lambda = 1.49 \text{ \AA}$  is selected by the reflection on a (111) vertical face of a Ge crystal. The incidence angle on the monolayer plane is  $1.9 \text{ mrad}$ , below the critical angle  $\theta_c = 2.44 \text{ mrad}$  for total external reflection on formamide. The theoretical resolution for in-plane transfer wavevector  $q_{xy}$  is of the order of  $0.01 \text{ \AA}^{-1}$  fwhm (full width at half maximum). A position sensitive detector analyzes the out-of-plane diffraction intensity, as a function of the vertical momentum transfer  $q_z$ . It uses xenon as detecting gas, and ethane as a moderator. The observable  $q_z$  window along the vertical direction is limited by the height of Soller slits placed in front of the detector: it can be either  $0 < q_z < 0.8 \text{ \AA}^{-1}$  (when the slits are in their lower position), or  $0.6 < q_z < 1.2 \text{ \AA}^{-1}$  (higher position).

Formamide (Merck, for analysis) is used without further purification. DSPC (Avanti Polar Probes) is spread from a solution in chloroform ( $\approx 10^{-3} \text{ M}$ ). The trough temperature is regulated at  $T = 10^\circ\text{C}$ . At

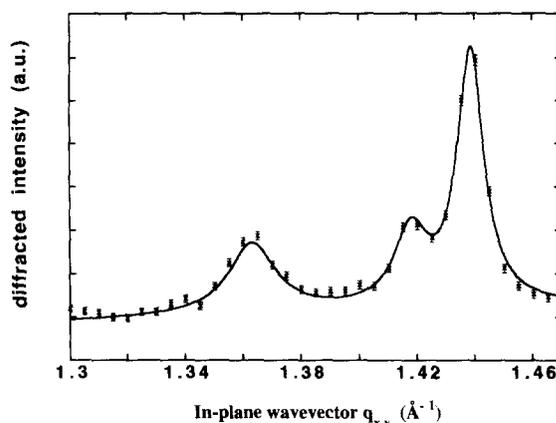


Fig. 1. Diffracted intensity (arbitrary units) for DSPC on formamide, versus in-plane wavevector  $q_{xy}$  ( $T = 10^\circ\text{C}$ ). The intensity, corrected for geometrical and polarization factors, is integrated over the window  $[0, 0.8 \text{ \AA}^{-1}]$  in the out-of-plane  $q_z$  direction. The best Lorentzian fit is also represented, showing the three peaks of the oblique structure.

this temperature, the DSPC isotherm presents a gas–solid coexistence plateau ( $A > 50 \text{ \AA}^2/\text{mol}$ ,  $\Pi \approx 0 \text{ mN/m}$ ), followed by a rapid pressure increase in the homogeneous solid phase. Evidence for a 2D solid behaviour in this phase comes from the rigidity of domains observed in fluorescence microscopy [4], and also from the anisotropy of surface stresses under directional compression, detected by two perpendicular Wilhelmy papers [7].

Diffraction spectra have been recorded as a function of the in-plane wavevector  $q_{xy}$ , integrated over  $0 < q_z < 0.8 \text{ \AA}^{-1}$ , at different surface pressures  $\Pi$  from 1 to 38 mN/m. Fig. 1 shows the scattered intensity at  $\Pi = 3.5 \text{ mN/m}$ , in the  $1.3\text{--}1.5 \text{ \AA}^{-1}$  region. The spectrum presents two well-separated peaks and a third one as a shoulder on the side of the most intense one. Following the procedure described in Ref. [9], the plotted intensity is corrected from bare data by a factor  $\tan^2(2\theta)$ , to account for the beam cross-section area correction  $\sin(2\theta)$ , the Lorentz factor  $\sin(2\theta)$  and the polarization factor  $1/\cos^2(2\theta)$ . The best Lorentzian fits, obtained with a maximum entropy algorithm (ABFit) are also drawn in Fig. 1. The peak positions (and fwhm) at  $\Pi = 3.5 \text{ mN/m}$  are, in  $\text{\AA}^{-1}$ : 1.363 (0.020); 1.418 (0.016); 1.439 (0.011). Notice that their width is comparable to the instrumental resolution. Sweeping a broad  $q_x$

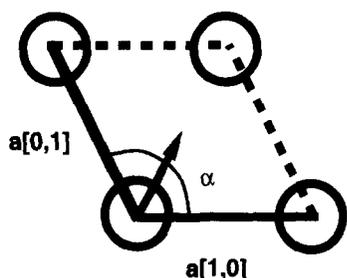


Fig. 2. Drawing of the oblique elementary cell for the chain lattice:  $a[1, 0] = 5.006 \text{ \AA}$ ,  $a[0, 1] = 5.207 \text{ \AA}$ ,  $\alpha = 117.74^\circ$ . The chain tilt (arrow) points towards the furthest site of the cell.

band, and especially looking for possible harmonics ( $q_{xy} \approx 2.5 \text{ \AA}^{-1}$ ) or subharmonics ( $q_{xy} \approx 0.8 \text{ \AA}^{-1}$ ), no other peak has been unambiguously detected.

These peaks correspond to a distorted but almost hexagonal lattice of aliphatic chains. At  $\Pi = 3.5 \text{ mN/m}$ , the oblique cell in real space is defined from Table 1 by  $a_{[1,0]} = 5.006 \text{ \AA}$ ,  $a_{[0,1]} = 5.207 \text{ \AA}$ , making an angle  $\alpha = 117.74^\circ$  (see Fig. 2). The third triangle side is  $a_{[1,1]} = 5.283 \text{ \AA}$ . The area per single chain is  $23.07 \text{ \AA}^2$ . Although there is no direct experimental evidence for order at the molecular level (a molecule has two chains), we believe that the solid phase is a crystal. The correlation length for positional order is given by the peak width, and should be comparable to the instrumental limitation, i.e.  $500 \text{ \AA}$ .

Several diffraction spectra have been recorded at various pressures, and they are all similar. Table 1 reports the peak positions and intensities as a function of  $\Pi$ . Several features should be underlined. First, apart from a small drift, the spectral shape and

the peak positions remain unchanged from 1 to 38 mN/m. Secondly, the average drift corresponds to a small change in area per molecule from  $A = 46.2 \text{ \AA}^2$  at 1 mN/m to  $45.7 \text{ \AA}^2$  at 38 mN/m. We infer the compression modulus  $K = 3500 \text{ mN/m}$ , which is large compared to similar condensed phases on water: for DSPC on water, one measures  $K = 500 \text{ mN/m}$  at  $15^\circ \text{ C}$  [10].

The intensity ratios of the different peaks vary from one run to another. We believe that this is due to deviations from an ideal powder spectrum. The typical domains size is known to be a few hundred micrometers [4], and their area is not negligible compared to the observed area  $\Delta S$  (a few  $\text{mm}^2$ ). Inside  $\Delta S$ , the number of domains is too small to be considered as a random powder.

The scattered intensity has been measured as a function of the vertical transfer wavevector  $q_z$ , for several  $q_{xy}$  around the three Bragg peaks. Typical Bragg rod scans are shown in Fig. 3, at  $\Pi = 3.5 \text{ mN/m}$ , for  $q_{xy}[1, 0] = 1.3625$ ,  $q_{xy}[0, 1] = 1.420$ ,  $q_{xy}[1, 1] = 1.440 \text{ \AA}^{-1}$ . The intensities measured in the two windows  $0 < q_z < 0.8$  and  $0.6 < q_z < 1.2 \text{ \AA}^{-1}$  are plotted on the same graph. Whenever necessary, they have been adjusted to each other by a normalization factor (after noise subtraction), to take into account the variations in the domain distribution from one scan to the other. Each plotted point is an average over nine detector channels.

While the  $q_{x3} = 1.440 \text{ \AA}^{-1}$  Bragg rod is centered on  $q_z = 0$ , the intensity  $I(q_z)$  for the two other  $q_x$  has a pronounced maximum around  $q_z = 0.85 \text{ \AA}^{-1}$ , meaning a non-zero chain tilt. Following Jacquemain et al. [11], we model the tilted molecule as a cylin-

Table 1

Pressure (mN/m)	$q_{xy}[1, 0]$ ( $\text{\AA}^{-1}$ )	Intensity (arb. un.)	$q_{xy}[0, 1]$ ( $\text{\AA}^{-1}$ )	Intensity (arb. un.)	$q_{xy}[1, 1]$ ( $\text{\AA}^{-1}$ )	Intensity (arb. un.)
1	1.364	68	1.417	53	1.435	97
3.5	1.363	40	1.418	34	1.439	80
15	1.369	47	1.420	83	1.437	100
20	1.370	51	1.421	43	1.440	160
30	1.380	63	1.427	72	1.447	139
38	1.371	12	1.426	11	1.443	78

drical rod of length  $L$ , and  $I(q_z)$  can be simply expressed as

$$I(q_z) = I_0 \left( \frac{\sin W}{W} \right)^2, \text{ where}$$

$$W = 0.5L [q_z \cos(t) - q_{xy} \cos(\psi_{xy}) \sin(t)]. \quad (1)$$

Here  $t$  is the chain tilt angle from the normal to the interface and  $\psi_{xy}$  is the azimuthal angle between  $q_{xy}$  and the in-plane tilt direction  $t$ . The best fit to the data (Eq. (1)) is also represented on Fig. 3. The fit parameters are:  $L = 25 \pm 1 \text{ \AA}$ ,  $t = 35 \pm 1^\circ$ ,  $\psi[1, 0] = 30.5 \pm 1^\circ$ ,  $\psi[0, 1] = -31.5 \pm 1^\circ$ ,  $\psi[1, 1] = 90^\circ$ . Other scans at slightly different  $q_{xy}$ , close to the peak maxima, can be fitted with the same values. This confirms that the film is a monolayer:  $L$  is consistent with other measurements on same chain length phospholipids [6]. The tilt points towards the furthest neighbouring chain in the elementary lattice cell (see Fig. 2). From  $t = 35^\circ$  and  $A/2 = 23 \text{ \AA}^2$  one finds  $A_c = (A/2) \cos(t) = 19 \text{ \AA}^2$  for the chain area in a close-packed state, as expected [12]. We have checked that the rod scan intensity looks similar at  $\Pi = 30 \text{ mN/m}$ . Thus the tilt is the same at all pressures. This is consistent with the weak variation observed for the area per molecule. We conclude that the solid phase has the same structure in the  $0 < \Pi \leq 38 \text{ mN/m}$  range.

In Fig. 3, apart from a non-linearity in the detector response at  $q_z = 0.18 \text{ \AA}^{-1}$ , one notices a clear secondary intensity maximum around  $q_z = 0.4 \text{ \AA}^{-1}$ , especially for the  $q_{xy} = 1.3625 \text{ \AA}^{-1}$  scan. We do not have a satisfactory explanation for this. For instance, fitting the data with a two box model for the electronic density in the chain does not give a significantly better agreement. Also, the existence of two different tilts in the structure seems unlikely. We do not believe either that the layer could be partially collapsed, since this usually leads to narrower peaks [6].

As a final comment, notice that the intensity plotted in Fig. 1 is only integrated over the  $0 < q_z < 0.8 \text{ \AA}^{-1}$  window, out of the intensity maximum at  $q_z = 0.85 \text{ \AA}^{-1}$  for the  $[0, 1]$  and  $[1, 0]$  peaks. This explains why the  $[1, 0]$  and  $[0, 1]$  Bragg peak intensities are on the average smaller than the  $[1, 1]$ .

### 3. Comparison with water substrate and conclusion

A lot of work deals with the structure of phospholipids on water (different chain lengths and polar

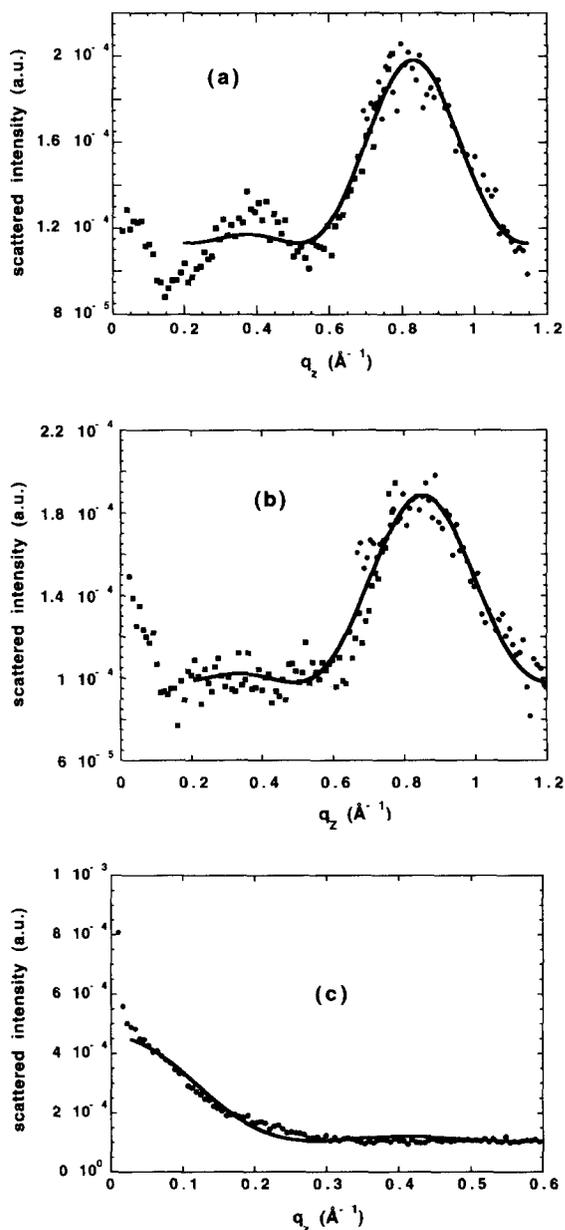


Fig. 3. Bragg rod scans (scattered intensity versus  $q_z$ ) at three wavevectors corresponding to the (a)  $[1, 0]$ , (b)  $[0, 1]$  and (c)  $[1, 1]$  peaks. The data in the range  $0 < q_z < 0.8 \text{ \AA}^{-1}$  ( $\blacksquare$ ) and  $0.6 < q_z < 1.2 \text{ \AA}^{-1}$  ( $\bullet$ ) are shown on the same graph, together with the fit corresponding to Eq. (1).

head). However, information concerning cholines on water has become available only quite recently.

X-ray diffraction performed on phosphatidylethanolamines spread on water show a tilted/untilted transition [6,13]. For DMPE, below  $\Pi_c = 25$  mN/m (at 20°C), the chain lattice is oblique, and the tilt decreases continuously to zero as  $\Pi_c$  is approached. Above  $\Pi_c$  the structure is hexagonal. The same phenomenon occurs for DSPE.

Concerning phosphatidic acid (DMPA) and phosphatidylcholines (DMPC, DPPC) the surface isotherms seem to present a transition (between 10 and 30 mN/m at room temperature, depending on the head group) [14], but recent work indicates that no tilted/untilted transition occurs for DHPC, DPPC and DSPC on water [10,15]. The structure of the condensed phase is oblique for DHPC and DPPC and rectangular for DSPC. The chain tilt decreases, but never vanishes in the 0–40 mN/m pressure range. Simultaneously, the area per molecule changes from about 45 to about 40 Å<sup>2</sup>/mol.

Then, the main difference with formamide comes from the compressibility of the crystalline phase. For DSPC on formamide,  $K = 3500$  mN/m at 10°C. On water,  $K = 500$  mN/m for DSPC, 350 mN/m for L-DPPC and 300 mN/m for L-DHPC, at 15°C. It is difficult to explain such a difference by temperature effects, and we can only speculate about the origins of this phenomenon. It has been suggested [16] that the phosphatidic group may have stronger interactions with highly dipolar formamide than with water, through the amine group. This could lead to a larger area per head and thus give rise to a stiffer tilted monolayer.

Among the consequences of this specific behaviour, we would like to point out that the recent observation of buckling in a DSPC monolayer on formamide [7], occurring above  $\Pi = 7$  mN/m, cannot be induced by any phase transition in the film: the film structure remains the same in a quite large range of surface pressure. It also seems likely that the high rigidity of this phase favors the occurrence of buckling.

Further work should complete these structural investigations, in the solid phase up to 19°C, and at

higher temperature where several mesophases have recently been discovered.

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