DIRECT OBSERVATION OF THE HYDROCARBON CHAIN TILT ANGLE IN PHOSPHOLIPID BILAYERS

J. B. STAMATOFF, W. F. GRADDICK, L. POWERS, AND D. E. MONCTON,
Bell Laboratories, Murray Hill, New Jersey 07974 U.S.A.

ABSTRACT By means of X-ray diffraction we have observed the tilting of hydrocarbon chains within a dipalmitoyl phosphatidylcholine multilayer. The tilting produces a distribution of intensity in the $(4.2\ \text{Å})^{-1}$ region that provides a quantitative measure of the angle of tilt. Detailed diffraction investigation of multilayers prepared by a recently developed method and mounted to form a "freestanding" sample has made possible the accurate measurement of intensity in this region. We find the intensity distribution to be different from that previously reported and proceed to develop a new model for tilted chains. The model permits calculation of the intensity distribution as a function of tilt angle which is then fit to the data.

INTRODUCTION

In 1973, Tardieu et al. (1) predicted tilting of hydrocarbon chains in phospholipid bilayers, and calculated the angle of tilt based on measurements determining the surface area of a phospholipid molecule. These results were obtained by X-ray diffraction of unoriented phospholipid multilayers, involving measurement of the multilayer periodicity and line shape of the $(4.2\ \text{Å})^{-1}$ ring. These findings supported earlier observations of Levine (2–4), who studied oriented multilayers prepared by the method of Langmuir and Blodgett. For samples containing 20% water by weight, he found that the $(4.2\ \text{Å})^{-1}$ reflection was distributed with peaks both on and off the equator, and interpreted this distribution as due to tilting of hydrocarbon chains. These studies (1–4) concluded that the chain tilt varied between 0° and 30°, depending on the water content of the dipalmitoyl phosphatidylcholine (DPPC) multilayer sample. By studying highly oriented and well-stacked "freestanding" samples of DPPC multilayers, we can accurately measure the distribution of intensity in the $(4.2\ \text{Å})^{-1}$ region in a manner which is free from many experimental complications. We find a different intensity distribution and proceed to calculate this distribution based on a new model of tilted chains subject to angular disorientation. This calculation provides a precise determination of tilt angle.

METHODS

Sample Preparation

We prepared our samples from pure DPPC (Calbiochem, San Diego, Calif., or Gateway Chemical Corp., Kansas City, Mo.) by the methods of Powers and Clark (5). Water was incorporated into the samples by submersion of the glass slide assembly into water above the gel transition (90 ± 5°C), or by directly adding the water with a microliter syringe before alignment (6). The sample preparation was carried out under N_2.

After sample alignment at room temperature, we determined the water content to an accuracy of

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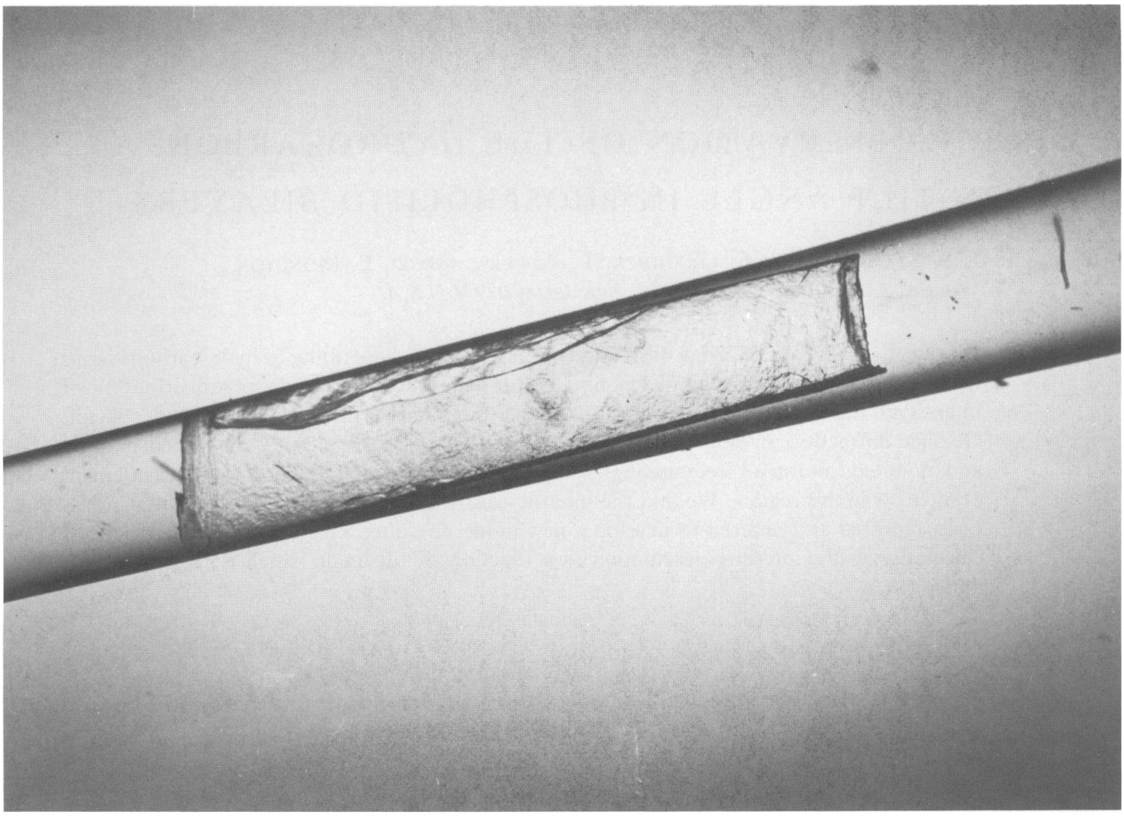

FIGURE 1 A DPPC multilayer sample mounted inside a quartz capillary tube for X-ray diffraction.

$\pm 1\%$ by measuring the birefringence which is correlated with water content by dry weight determinations (6). In certain cases excess sample (from the same slide), which had not been used for diffraction, was used for a dry weight determination. Samples used in this study were optically biaxial.

For diffraction, we separated the oriented specimens at room temperature from the glass slides and sealed them inside thin-walled quartz capillaries under N_2. Typical dimensions were 1 mm × 3 mm × 0.1 mm. A photograph of a sample inside a capillary is shown in Fig. 1. The thin dimension is along the stacking direction.

This method of lipid multilayer preparation (5) produces well-oriented samples. Water is incorporated above the gel transition. However, the diffraction experiments were performed after the sample was allowed to cool and was well below this transition. Therefore, these measurements apply to oriented DPPC multilayers in the gel phase.

Nomenclature

We define $\mathbf{C}^*$ as the reciprocal space vector which is along the stacking direction (perpendicular to the bilayer plane). $C^* = |\mathbf{C}^*|$. We define $\mathbf{A}^*$ as a vector orthogonal to $\mathbf{C}^*$ and therefore parallel to the plane of the bilayer. $A^* = |\mathbf{A}^*|$. The A^*C^* plane is perpendicular to the bilayer plane. A^* and C^* are measured in units of $2\sin\theta/\lambda$, where 2θ is the scattering angle and $\lambda = 1.54$ Å.

For comparison with previous investigations (2–4), $\mathbf{C}^*$ corresponds approximately to the meridian as recorded with film. $\mathbf{A}^*$ corresponds approximately to the equator.

Experimental Method

Data were collected at room temperature (22 ± 3°C) by using a three crystal spectrometer, in which the angle (ϕ) of the sample with respect to the beam and the angle (2θ) of the detector may be varied independently. A bent pyrolytic graphite (PG) monochromator crystal focused X-rays perpendicular to the diffraction plane and produced a spot at the sample of $\sim 2\ \text{mm}^2$. A flat graphite analyzer crystal, used after the sample, decreased stray background. X-rays were detected using a NaI scintillation. Both PG crystals have mosaic spreads of approximately 0.4°. We used copper Kα X-rays obtained from 50 kW rotating anode generator.

RESULTS

We studied samples containing 2 ± 1% and 10 ± 1% water by weight. The result of a scan along C^* for a sample containing 2% water by weight (a 2% H_2O sample) is shown in Fig. 2. The large number of lamellar reflections recorded indicates the degree of order within the sample and the signal-to-background ratio of the spectrometer. For 2% H_2O samples, the periodicity along C* is $(55.7\ \text{Å})^{-1}$, and for 10% samples it is $(57.4\ \text{Å})^{-1}$.

The intensity of the fourth lamellar reflection for a 2% H_2O sample versus the angle, ϕ, (at constant 2θ) of the sample, with respect to the beam (shown in Fig. 3), gives a mosaic spread of 4° (full width at half maximum [FWHM]). Although various samples had different mosaic spreads, each was less than 15°. This curve measures the angular distribution of intensity in the A^*C^* plane. It is a direct measurement of the angular distribution of crystallites about the stacking direction and within a plane perpendicular to the bilayer. The samples are highly disordered about the stacking direction within the plane of the bilayer.

Many scans were performed in the A^*C^* plane. A grid of points was assembled and equal

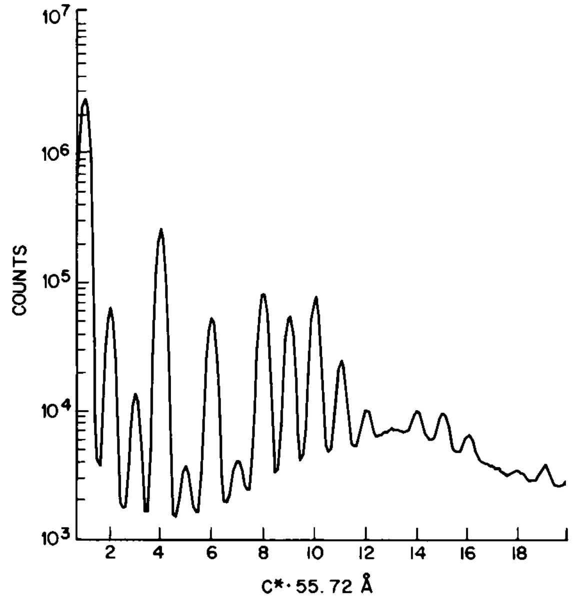

FIGURE 2 Lamellar reflections for a 2% H_2O DPPC multilayer sample. This is a scan along C*. The periodicity is 55.72 Å.

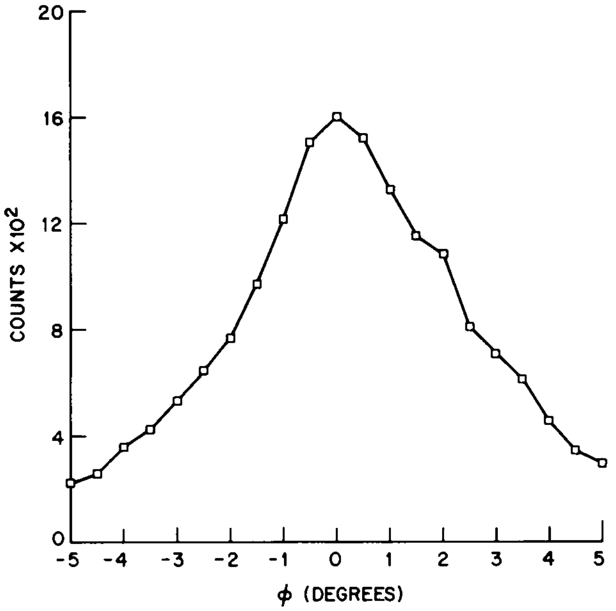

FIGURE 3 The intensity of the fourth lamellar reflection for a 2% H_2O sample versus the angle ϕ. This curves gives a mosaic spread of 4°.

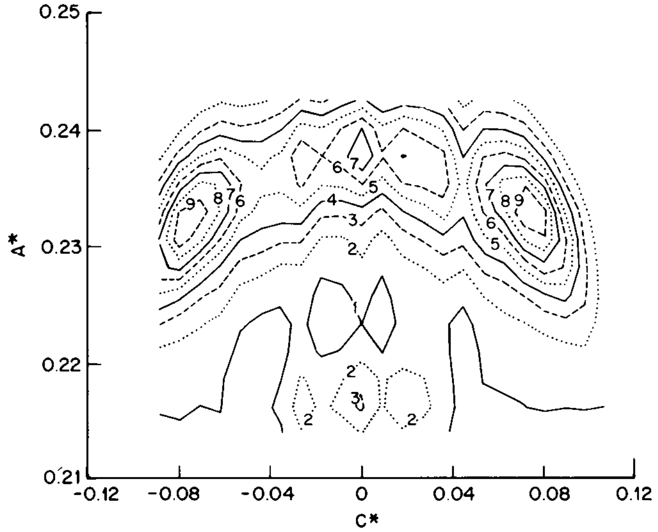

FIGURE 4 Intensity contour map of the (4.2 Å)$^{-1}$ region for a 2% H_2O sample. The mosaic spread of this sample is 4°. The levels are indicated by number and correspond to the following number of counts: (*1*) = 600; (*2*) = 800; (*3*) = 1,000; (*4*) = 1,200; (*5*) = 1,400; (*6*) = 1,600; (*7*) = 1,800; (*8*) = 2,000; (*9*) = 2,200.

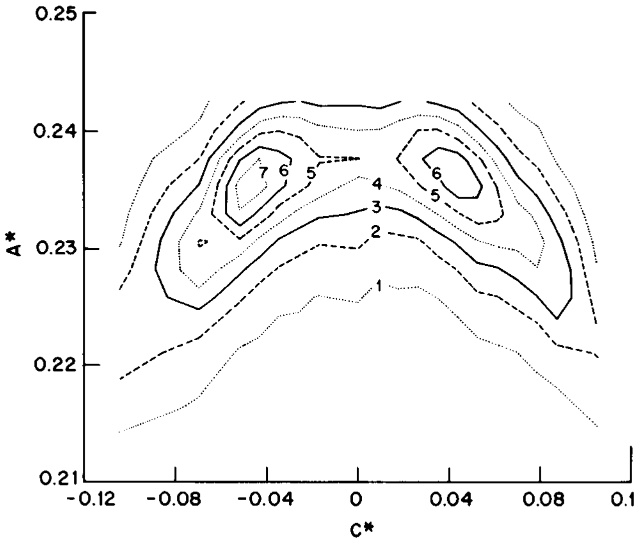

FIGURE 5 Intensity contour map of the (4.2 Å)$^{-1}$ region for a 10% H_2O sample. The mosaic spread of this sample is 10°. The levels are indicated by number and correspond to the following number of counts: (*1*) = 600; (*2*) = 800; (*3*) = 1,000; (*4*) = 1,200; (*5*) = 1,400; (*6*) = 1,600; (*7*) = 1,800.

intensity contours were drawn. An example of such a grid is shown in Fig. 4 for a 2% H_2O sample. Unlike previous results for monohydrate multilayers (2–4), we find that there are many intensity maxima.

We also find that the intensity distribution changes with water concentration. Fig. 5 shows our results for a 10% H_2O sample. Repetition of these results for several 10% H_2O samples shows that the intensity forms a cylindrical surface about C* with maxima above and below the A* axis. We observe the intersection of this surface with the A^*C^* plane, which forms two line segments of modulated intensity with maxima near the ends. Unlike previous results (2–4), we do not find six maxima but only four (two on either side of the origin; the intensity distribution is centrosymmetric). Specifically, we do not find maxima at $C^* = 0$ (on the equator) in the (4.2 Å)$^{-1}$ region.

In both Figs. 4 and 5, the scale is expanded along A^* to display the features. A plot in which A^* and C^* are on the same scale shows that the intensity more closely approximates a line segment than an arc. This feature has also been determined by Buerger precession photographs. Such photographs show, in addition, no other maxima outside the region displayed in Figs. 4 and 5. In contrast, previous results indicate that the intensity occurs in arcs equidistant from the origin.

ANALYSIS

First we consider the higher-water content samples. Qualitatively, the major discovery is the absence of maxima on the equator (C* = 0) and presence of maxima above and below it. If we accept an hexagonal or quasi-hexagonal lattice, it is impossible to produce only four maxima by tilting the hydrocarbon chains. We concur with Levine's (2–4) observation that there should be six peaks. Therefore we develop a new model in which the hydrocarbon chains may rotate in any direction with respect to the hexagonal lattice, at a fixed tilt angle with respect to the bilayer plane. This model reproduces the main features of the observed intensity distribution. In addition, the maxima fall along a line, as our data suggest. We are unable to determine by our methods whether the rotation of tilted chains is merely a static superposition of many crystalline conformations or a dynamic process within each mosaic.

We consider the consequence of placing long hydrocarbon chains on a two-dimensional lattice (e.g., a hexagonal lattice) in the plane of the bilayers. For the 10% H_2O case, it is assumed that bilayers can translate freely with respect to one another while maintaining their stacking order. The reciprocal lattice consists of lattice lines parallel to **C***. The intensity distribution is obtained by calculating the square of the product of these lattice lines with the Fourier transform of a single tilted hydrocarbon chain. For simplicity, this transform may be approximated by a plane orthogonal to the long axes of the hydrocarbon chain. Therefore, peaks of intensity will be confined to this plane which is tilted with respect to the bilayer plane.

We assume that our sample is composed of crystallites which are unoriented about the stacking direction within the plane of the bilayer. The sum of lattice lines from the individual mosaics forms concentric cylinders with **C*** as the axis. For this analysis, we will consider only one cylinder with radius $(4.2\ \text{Å})^{-1}$.

For chains independently disoriented about the stacking direction, but which maintain a fixed angle of tilt with respect to the bilayer plane, the intensity is found by forming the product of the cylinder with a tilted plane. This forms an ellipse as shown in Fig. 6. The intensity, dI, for each angular increment, $d\beta$, about **C***, is a constant, $C_0 = dI/d\beta$, for a perfectly disordered system.

In the experimental frame, the ellipse describes the intensity for one chain orientation.

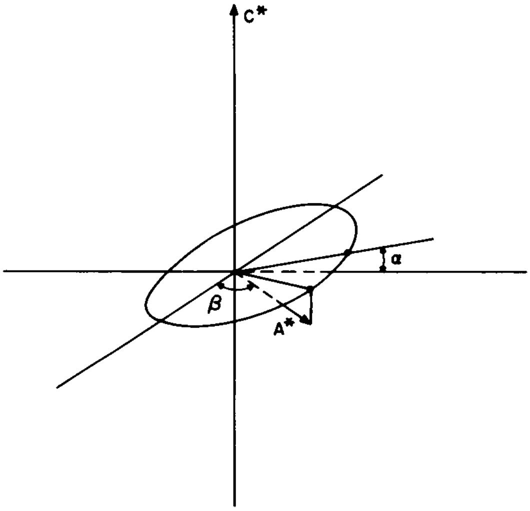

FIGURE 6 Elliptical envelope of intensity produced by the intersection of a tilted plane with lattice lines randomly oriented about **C***. To produce the observed intensity, this ellipse is allowed to precess about **C***.

To account for chain disorientation about the stacking direction, the ellipse of Fig. 6 must precess about **C***. The result is a cylindrically symmetric surface.

We measure the intensity on the cylindrical surface along a line parallel to **C*** at a constant value of $A^* \sim (4.2\ \text{Å})^{-1}$ experimentally. The intensity, dI, for an interval, dC^*, along this line is given by

$$\frac{dI}{dC^*} = \frac{dI}{d\beta}\,\frac{d\beta}{dC^*} = \frac{C_0}{A^* \cos\beta \tan\alpha}$$

or

$$\frac{dI}{dC^*} = \frac{C_0/A^* \tan\alpha}{\left[1 - \dfrac{C^{*2}}{A^{*2}\tan^2\alpha}\right]^{1/2}}.$$

The above equation approximates the Fourier transform of the hydrocarbon chain as a plane. This approximation would be accurate only if the hydrocarbon chains were infinitely long and thin. The calculation may be improved by considering, instead, a rod of length l_0 and radius r_0. Following Hui (7), the transform is given by

$$F = F_0\,\frac{J_1(2\pi r^* r_0)}{\pi r^* r_0}\,\frac{\sin 2\pi l^* l_0}{\pi l^* l_0},$$

where r is orthogonal to the rod, l^* is parallel to the rod, and F_0 is a constant.

Tilting this rod produces an elliptical surface similar to that depicted in Fig. 6, but extended parallel to C^*. For small angles of tilt, $r^* \approx A^*$, which is a constant on the surface. In addition,

$$C^* = \frac{l^*}{\cos\alpha} + A^* \sin\beta \tan\alpha.$$

Therefore

$$I \approx F_1^2 \left[\frac{\sin 2\pi l_0(C^* \cos\alpha - A^* \sin\beta \sin\alpha)}{\pi l_0(C^* \cos\alpha - A^* \sin\beta \sin\alpha)}\right]^2,$$

where

$$F_1 = F_0\,\frac{J_1(2\pi A^* r_0)}{\pi A^* r_0}.$$

For tilted chains which are free to rotate about **C*** independently of the rotation of the lattice lines, one obtains.

$$I \approx K \int_0^{2\pi} \left[\frac{\sin 2\pi l_0(C^* \cos\alpha - A^* \sin\beta \sin\alpha)}{\pi l_0(C^* \cos\alpha - A^* \sin\beta \sin\alpha)}\right]^2 d\beta,$$

where K is a constant.

Fig. 7 is a plot of the above equation with data from a scan parallel to **C*** at $A^* = 0.2354$ Å^{-1}. For this curve, the best fit occurs at a tilt angle of ~ 12.5°.

We have assumed that both the lattice and tilted chains have, independently, 360° mosaic

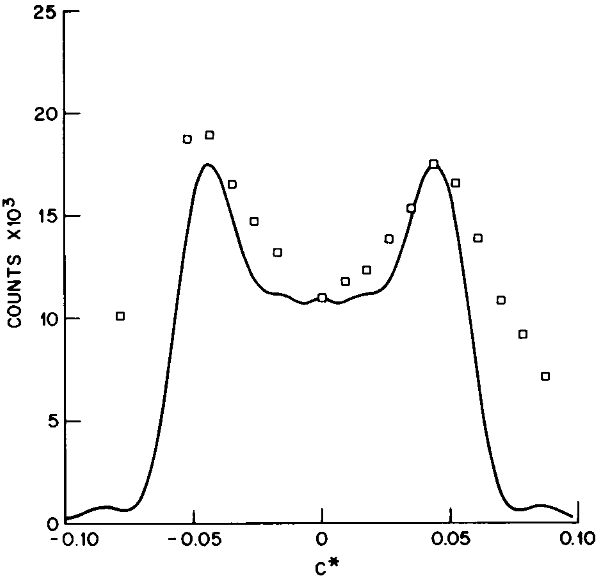

FIGURE 7 Model (solid line) versus actual intensity for a 10% sample. The scan is parallel to C* at $A^* = 0.2354$ Å^{-1}. The model is for rods of length 20 Å that are tilted 12.5°.

spreads about C* within the plane of the bilayer. Similar intensity distributions would be observed for several lattice lines at the same distance A^* from the C* axis (e.g. an hexagonal lattice), where the mosaic spreads were smaller. This possibility allows for the weak optical biaxiality of these samples.

At lower water content, the 2% sample shows more intensity maxima in the (4.2 Å)$^{-1}$ region. There are two obvious possibilities. First, the tilted chains may be restricted to rotate with the lattice lines as previously suggested (2-4). The result would be a series of equal diameter rings about C*, whose placement along C* depends upon the type of lattice and direction of tilt within the lattice. Second, bilayer-to-bilayer correlations may develop as the stacked sample approaches a three-dimensional crystal, so the lattice lines would be multiplied not only by the transform of a tiled molecule, but also by a set of fringes due to this correlation.

DISCUSSION

Using unoriented samples of DPPC, Tardieu et al. (1) analyzed the line shape of the (4.2 Å)$^{-1}$ reflection. The changes in the profile were interpreted as tilting of the hydrocarbon chains in a distorted hexagonal lattice. Our results for the 10% H_2O sample of DPPC are a direct experimental measure of this tilting.

Our measurement of stacking periodicity for the 10% H_2O sample is 57.4 Å, which compares favorably with 57.0 Å measured by Tardieu et al. (1) for an 11% H_2O sample. The value of 12.5°, which we determine for the tilt by using this direct method with oriented samples, is smaller than that previously calculated.[1]

In comparison with previous work on oriented wet samples (2-4), we do not observe maxima at $C^* = 0$ in the (4.2 Å)$^{-1}$ region (on the equator). In previous work, a backing block was used to support bilayers, and difficulties associated with the method have been discussed (2). Our method has the virtue of eliminating the supporting block and using a diffractometer, so the beam remains roughly parallel to **C***. Thus we diminish absorption effects due to the sample and eliminate absorption and scattering from a supporting block. These problems are maximized on the equator and probably account for the observed differences.

The advantages of our experimental technique are not easily applicable to samples containing considerably greater than 10% water. These samples are readily deformed and would require, for study via diffractometry, the use of an X-ray transparent supporting block.

Accurate measurement of intensity in the $(4.2\ \text{Å})^{-1}$ region requires samples possessing small mosaic spreads. For the 10% H_2O sample the mosaic spread is ~10° (FWHM). Thus a scan parallel to **C*** at $A^* = 0.2354\ \text{Å}^{-1}$ has a resolution due to mosaic spread of approximately $0.04\ \text{Å}^{-1}$. Therefore maxima at $C^* = 0$ should be observed. At 2% H_2O a mosaic spread of 4° produced a resolution for a similar scan of less than $0.02\ \text{Å}^{-1}$.

The absence of an equatorial maximum has led us to propose a new model in which hydrocarbon chains and the lattice on which they are placed rotate independently about **C***. The chains maintain a given angle of tilt for all orientations with respect to the lattice. This model permits calculation of the intensity distribution in the $(4.2\text{Å})^{-1}$ region. The obtained value of 12.5° for the tilt angle of a 10% sample is expected to increase with water content (1–4). The range of applicability of our model in both temperature and water content is undetermined. Obviously this model would not apply above the gel transition.

At lower water content we find many diffraction maxima. Our measured periodicity for 2% H_2O is 55.7 Å. Previous results (4) for 2% H_2O samples do not show the many features we observe. However, the measured periodicity of 57 Å (4) indicates that the difference may be simply due to determination of water content.

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