

Chapter 2

Data Analysis

2.1 Introduction

As discussed in chapter-I, x-ray **diffraction** and freeze fracture electron microscopy are the two techniques commonly used to probe the equilibrium structure of the ripple ($P_{\beta'}$) phase of phospholipids. We have used the x-ray diffraction method to determine electron density maps of the ripple phase for different lipids and lipid mixtures. An electron density map (EDM) gives the local density of electrons (and hence atoms) that scatter x-rays within a unit cell [1]. We have analyzed data mainly from four sources: (1) data from unoriented sample of DMPC obtained using a synchrotron source, reported by Wack and Webb in ref. [2], (2) data from oriented samples of DMPC and POPC taken by Raghunathan and Katsaras [3] using a rotating anode x-ray generator, (3) data from oriented samples of DHPC and mixtures of DPPC and DHPC taken in our own laboratory again using a rotating anode generator and (4) data from unoriented samples of DLPC reported by Tardieu et. al. in ref. [4]. Other data available in the literature have too many overlapping **reflections** to be suitable for this kind of analysis [5]. In this chapter, we first give **an** introduction to x-ray diffraction. In section-2.2 we discuss the details of the experimental procedure and in section-2.3 the procedure for data reduction, with **special** reference to the problem of applying absorption correction to data from samples aligned in the geometry specific to our experiment. The modeling and fitting procedure used to calculate the phases of the reflections is described in sections-2.4 and -2.5. The electron density maps

obtained by this procedure will be discussed in chapter-111.

2.1.1 Diffraction and Fourier transform

The scattering of x-rays by a single electron can be described by Thompson scattering where the electron is free and the wavelengths of the incident and the scattered radiations are the same. If the electric field associated with the incident radiation is E_{in} , then the electric field associated with the scattered wave can be written as $E_{scat} = f_e E_{in}$; f_e is called the electronic scattering factor and represents the ability of the electron to scatter radiation. It depends on fundamental constants and is an intrinsic property of electrons.

The scattering from an array of n electrons results in vector addition of the electric fields associated with the radiation scattered from each electron $E_{scat} = \sum_n f_e e^{i\Phi_n} E_{in}$, where Φ is a phase factor that is determined by the relative positions of the electrons and the wavelength of the incident wave. For the j^{th} electron, $\Phi_j = \mathbf{q} \cdot \mathbf{r}_j$, where $\mathbf{q}$ is the reciprocal lattice vector and $\mathbf{r}_j$ the position vector of the electron. In case of a continuous distribution of electrons, the scattering by a volume element is given by $dE_{scat} = f_e \rho(\mathbf{r}) d\mathbf{r}$ where $\rho(\mathbf{r})$ is the density of electrons, $\rho(\mathbf{r}) d\mathbf{r}$ is the number of electrons in the volume element $d\mathbf{r}$. Therefore the total scattered amplitude is given by

$$F(\mathbf{q}) = \int_{\text{all } \mathbf{r}} f_e \rho(\mathbf{r}) e^{i\mathbf{q} \cdot \mathbf{r}} d\mathbf{r}$$

Thus it is clear that the scattered amplitude from an object whose structure is described by a function $\rho(\mathbf{r})$, is the Fourier transform $F(\mathbf{q})$ of $\rho(\mathbf{r})$. In case of x-rays, the electrons are the scattering centers and therefore $\rho(\mathbf{r})$ describes the distribution of electrons in space. The structure of a periodic object (a crystal or a liquid crystal for example) can be described by a convolution of the **structure** of the lattice $[\rho(\mathbf{r})]_{lattice}$ with the structure of the basis $[\rho(\mathbf{r})]_{basis}$:

$$\rho(\mathbf{r}) = [\rho(\mathbf{r})]_{lattice} * [\rho(\mathbf{r})]_{basis}$$

Therefore, in Fourier space, the Fourier transforms are related by:

$$F(\mathbf{q}) = [F(\mathbf{q})]_{lattice} \cdot [F(\mathbf{q})]_{basis}$$

In real space,

$$[\rho(\mathbf{r})]_{lattice} = \sum_{n_1, n_2, n_3} \delta(\mathbf{r} - [n_1 \mathbf{a} + n_2 \mathbf{b} + n_3 \mathbf{c}])$$

where $\mathbf{a}$, $\mathbf{b}$ and $\mathbf{c}$ are the basis vectors and n_1 , n_2 and n_3 are integers. Therefore

$$[F(\mathbf{q})]_{lattice} = \sum_{h, k, l} \delta(\mathbf{q} - (h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*))$$

where $\mathbf{a}^*$, $\mathbf{b}^*$ and $\mathbf{c}^*$ are the basis vectors in the reciprocal space and h, k, l are integers.

The form factor $[F(\mathbf{q})]_{basis}$ is determined by the **scattering from** the contents of one unit cell. The amplitude of the diffraction pattern from the crystal, called the structure factor, is the form factor of the unit cell sampled at the reciprocal lattice points $\mathbf{q}_{hkl} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*$. It is given by

$$F_{hkl} = \int_{\text{unit cell}} \rho(\mathbf{r}) e^{i\mathbf{q}_{hkl} \cdot \mathbf{r}} d\mathbf{r} \quad (2.1)$$

2.1.2 The phase problem

In an x-ray experiment, only the intensities ($|F_{\mathbf{q}}|^2$), and not the structure factors ($F_{\mathbf{q}}$) themselves are recorded. But $|F_{\mathbf{q}}|^2 = |F_{\mathbf{q}} e^{i\Phi}|^2$ where Φ can be **any** real number. For a centrosymmetric object, $\rho(\mathbf{r}) = \rho(-\mathbf{r})$. Thus, when the Fourier integral is calculated, the imaginary part of the integrand is an odd function and integrates to zero whereas the real part is an even function and contributes to the Fourier transform. Therefore the Fourier transform of a centrosymmetric function is real and scattering from a centrosymmetric object gives rise to $e^{i\Phi} = \pm 1$.

The information about the phase Φ is lost when only intensities are recorded. The major challenge in determination of crystal structure from x-ray data involves the determination of the phases of the reflections. Once the phases are known, the electron density can be reconstructed by inverting eq.-2.1; that is, by taking the inverse Fourier transform of $F(\mathbf{q})$:

$$\begin{aligned}\rho(\mathbf{r}) &= \sum_{\mathbf{q}} F(\mathbf{q}) e^{-i\mathbf{q}\cdot\mathbf{r}} \\ &= \sum_{h,k,l} F_{hkl} e^{-i\mathbf{q}_{hkl}\cdot\mathbf{r}}\end{aligned}\tag{2.2}$$

2.2 Experimental procedure

2.2.1 Temperature and humidity control:

A locally built temperature and humidity controlled heater was used to collect data from oriented samples of DHPC, DPPC and their mixtures. The heater consists of an air tight nickel-plated brass chamber with a double wall through which water can be circulated. Temperature of the heater is controlled (to within $\pm 0.5^\circ\text{C}$) by circulating suitably heated (or cooled) water. The chamber has an entrance and an exit window for the x-rays. These windows are covered by mylar sheets. A humidity and temperature sensor (Testo **610**) is introduced into the chamber to monitor these parameters. A small electric fan is placed inside to induce air flow and thus keep the humidity and temperature inside as uniform as possible. The humidity is maintained by placing a saturated salt solution inside the chamber. The salt solution equilibrates with the atmosphere inside the chamber and thus the humidity is maintained (to within $\pm 2\%$) at a value fixed by the specific salt solution used. A schematic diagram of the heater is given in figure 2.1.

While working at temperatures more than a few degrees above the room temperature, moisture condensation on the inner surface of the windows **which** are at a slightly lower temperature has to be prevented by heating them from outside. This moisture condensation disturbs the humidity **balance** inside the chamber. The heating is done by blowing warm air onto the mylar windows from outside.

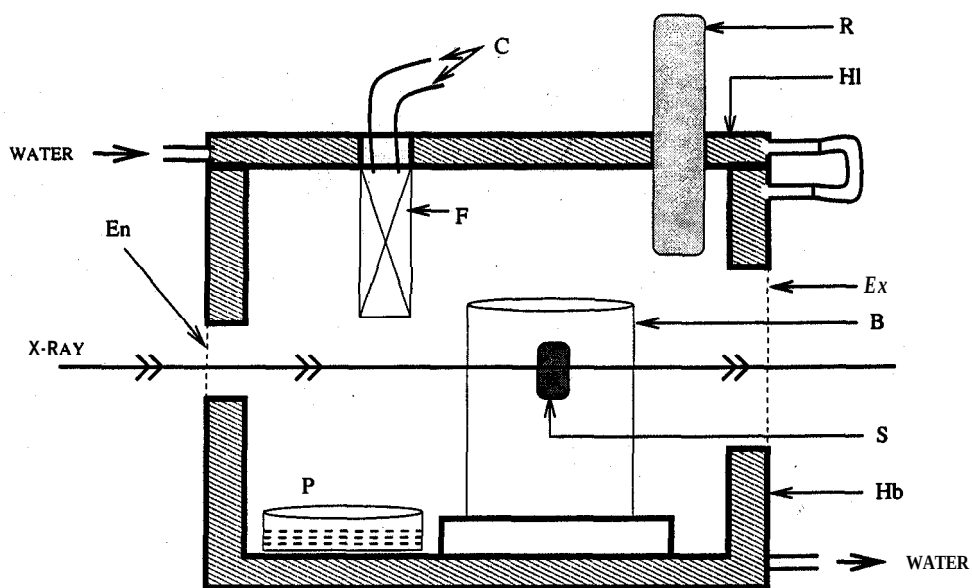


Figure 2.1: A schematic diagram of the humidity controlled heater used to collect data from aligned samples. Hl and Hb : the lid and the body of the heater, the double walls through which temperature controlled water is circulated is indicated by shading. En and Ex: the entrance and exit windows for the x-ray - both covered with mylar sheets. R: the temperature and humidity sensor. B: beaker on which the sample is deposited. S: deposited sample. P: petri-dish with salt solution to control the humidity inside. F: fan to circulate air. C: electrical connections for the fan.

2.2.2 Sample preparation

All the lipids studied were obtained from *Sigma chemical Co.* and were used as received. The sample is prepared by dissolving desired amounts of the lipid in a solvent (methyl alcohol in our case) and then spraying a little of the solution on the convex surface of a glass beaker (radius ≈ 1.5 cm). The solvent is then evaporated off by placing the beaker in an evacuated dessicator for about one hour. To hydrate the sample, it is sealed in an air tight container along with some pure water for about two days. A relative humidity (RH) of about $98 \pm 2\%$ can be achieved in our heater by this method. A suitable salt solution is used instead of pure water if a humidity less than $\approx 98\%$ is required. The sample is cycled up and down through the main and pre transitions a few times to facilitate hydration and alignment. A stack of bilayers, all oriented parallel to the curved glass surface is obtained by this method.

An unoriented sample consists of many randomly oriented domains within each of

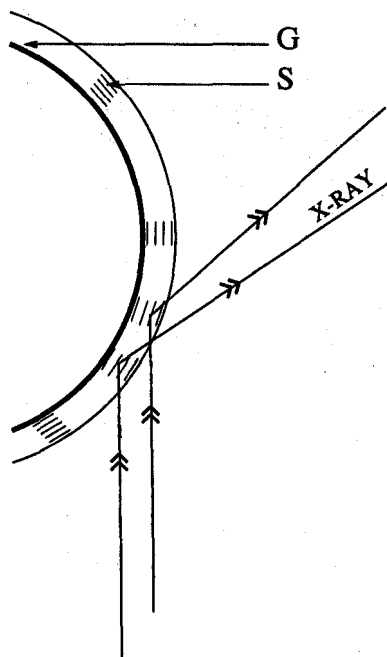


Figure 2.2: **The geometry of the sample.** G: glass surface of the beaker; S: deposited sample. The bilayers (indicated by set of parallel lines) are oriented parallel to the glass surface. In different parts of the sample, the bilayers make different angles with respect to the incident beam and hence different parts of the sample diffract into different Bragg angles.

which there is a high degree of positional and orientational correlation. The present method of preparing the sample orients the bilayers parallel to the substrate, but the ripple direction does not get fixed. This means that the domains are oriented at random with respect to the ripple direction but are all correlated with respect to the layering direction. Therefore the sample is oriented along the layer normal but is unoriented in the plane perpendicular to the layer normal.

Now, in order to sample more than one Bragg spot, normally an oriented sample has to be rotated while being exposed to x-rays. By depositing the sample on a curved surface, we get rid of the problem of rotating the sample. In this case, the curvature of the orienting surface introduces artificially the mosaicity that is necessary in order to scan many reflections simultaneously. But this means that different parts of the sample diffract into different Bragg-spots (see figure-2.2) and hence the absorption-correction (discussed in section-2.3.3) for each reflection is different.

2.2.3 Data collection

Cu K_α ($\lambda = 1.54 \text{ \AA}$) radiation from a rotating anode x-ray generator (*Rigaku, UltraX* 18) operating at 50kV and 80mA is used to illuminate the sample. A flat graphite monochromator (Huber) is used to make the incident x-rays monochromatic. The data is collected by an image plate (Marresearch, diameter 180 mm) which is connected to a computer with its own built-in software for data collection. Typical sample to film distance is about 200 mm and typical exposures last for about five hours. The images are obtained as gray scale plots of the intensity and are in the form of binary files. They have to be first converted to a form readable by standard softwares. We use the general purpose mathematical software package '**matlab**' to read the intensities. The image is displayed using '**matlab**' and the spot to be integrated is identified. A box is drawn around the spot and the contents of this box is redisplayed separately. The local contribution from the background is calculated by finding the average intensity of the pixels just outside the extent of the spot. The box should be large enough to contain all the high intensity pixels corresponding to the spot in question but not so large that it includes contributions from the neighboring spots. The average background intensity is subtracted from each pixel in the box and then the intensities of all the pixels inside the box are added up to get the total integrated intensity. The precise extent of the box is not important as long as the background subtraction is done properly. For the DHPC diffraction patterns, the binary files were directly displayed and manipulated by a locally written program that uses the image handling routines 'pg-plot' to display the image.

2.3 Data reduction

2.3.1 The unit cell

In the $P_{\beta'}$ phase, the ripples in the different bilayers are in registry and thus form a two dimensional oblique lattice (figure-2.3). The unit cell vectors are given by:

$$\mathbf{a} = d \cot \gamma \hat{x} + d \hat{z}$$

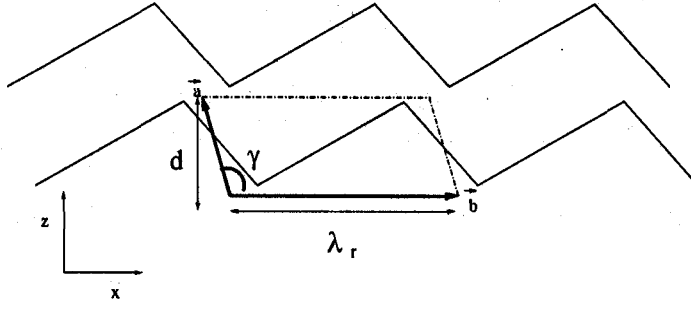


Figure 2.3: **Real space unit cell for asymmetric ripples.**

$$\mathbf{b} = \lambda_r \hat{x}$$

where λ_r is the ripple wavelength, d is the lamellar spacing and γ is the oblique angle. The lattice vectors in the reciprocal space are therefore given by:

$$\mathbf{A} = \frac{2\pi}{d} \hat{q}_z$$

$$\mathbf{B} = \frac{2\pi}{\lambda_r} \hat{q}_x - \frac{2\pi}{\lambda_r \tan \gamma} \hat{q}_z$$

where $\hat{q}_x$ and $\hat{q}_z$ are vectors in the reciprocal space corresponding to the real space vectors $\hat{x}$ and $\hat{z}$. The angle between the basis vectors in the reciprocal lattice is $\pi - \gamma$.

Therefore the q -space vector $\mathbf{q}_{hk}$ for the Bragg peak with Miller indices (h, k) is:

$$\begin{aligned} \mathbf{q}_{hk} &= h\mathbf{A} + k\mathbf{B} \\ &= \frac{2\pi k}{\lambda_r} \hat{q}_x + \left(\frac{2\pi h}{d} - \frac{2\pi k}{\lambda_r \tan \gamma} \right) \hat{q}_z \end{aligned} \quad (2.3)$$

In the above discussion, the rippling direction is the $\hat{x}$ direction and the bilayer stacking is along $\hat{z}$ direction. In that case, the spots along q_x (ie. the $(h, 0)$ reflections) correspond to the layer spacing and are called the main reflections. The other spots (with $k \neq 0$) arise due to the ripples and form an “X”-pattern around the main reflections, as the rippling direction is not oriented. These are called satellite reflections or simply satellites (a typical diffraction pattern is given in **figure-2.4**). The ripple-wavelength (λ_r), the bilayer repeat distance (d) and the oblique angle (γ) can thus be directly read off from the **diffraction** pattern. To get the other more subtle characteristics of the ripple profile; for example the actual shape, the sawtooth **am-**

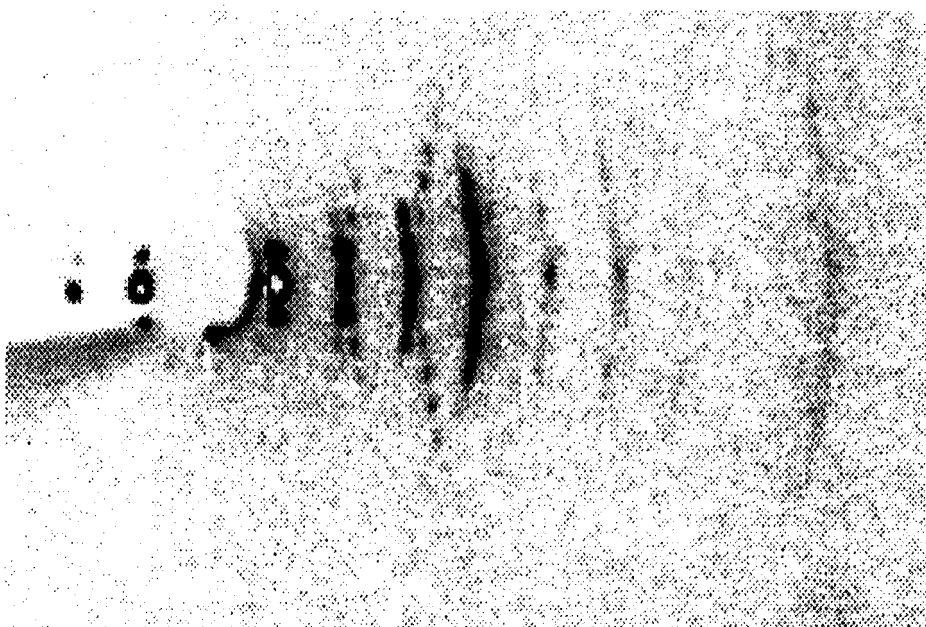


Figure 2.4: **A typical diffraction pattern from the ripple phase (DMPC).**

plitude and the bilayer thickness etc., the electron density map has to be calculated.

2.3.2 Geometric corrections

In a real system, because of various effects like finite extension of the sample, the finite angular spread of the incident beam coupled to the mosaicity of the sample, etc., the reciprocal lattice points are not mathematical points but are smeared out patches. In an experiment, the intersection of this smeared-out reciprocal lattice point with the Ewald sphere is recorded. To facilitate this intersection, the sample is usually either rotated or is taken in the form of randomly oriented crystallites so that the Bragg spots become arcs or rings. In the present case the sample is deposited on a curved surface and this increases the effective mosaicity of the sample in the direction of curvature and the Bragg spots become arcs that **subtends** an angle A at the origin of the reciprocal space. Since A is the same for all the spots, the smearing-out is more for larger scattering angles (see figure-2.5). This leads to a large reduction in the observed intensity of the reflections. To correct for this effect, the observed intensity has to be multiplied by the length of the arc which is given by $\frac{4\pi}{\lambda} \sin\theta \Delta$, where λ is the wavelength of the incident x-rays and θ the corresponding Bragg angle. This arc

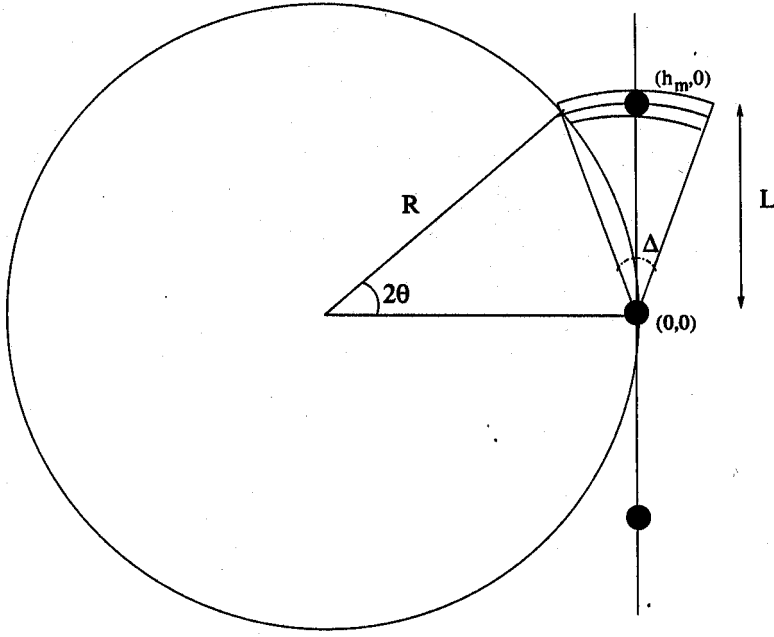


Figure 2.5: **Geometrical correction for the main reflections.** $(h_m, 0)$ is the highest order reflection detected. The circle of radius $R = \frac{2\pi}{\lambda}$ represents the Ewald sphere. The angular spread of the $(h, 0)$ reflections is Δ . This corresponds to a linear spread of $L\Delta$ where L is the distance (in reciprocal space) between the m^{th} order and the origin; $L = 4\pi \sin(\theta)/\lambda$. From simple geometric constructions, the condition for the m^{th} order reflection, corresponding to a Bragg angle θ_m , to intersect the Ewald sphere is: $\Delta = 2\theta_m$. To estimate L , it is assumed that the angular spread in the $L_{\beta'}$ phase and the $P_{\beta'}$ phase are the same. The highest order reflection visible in the $L_{\beta'}$ phase is the 8th order. This gives a lower limit for L since it is possible that the higher order reflections are present but are too faint to be detected. Another way of estimating L is from the fact that for the highest order recorded, the angle Δ is given by $\cos\Delta = (r - w)/r$ where r is the radius of the beaker and w is the beam width ($\times 1$ mm). The two estimates are comparable and typically, $\Delta \approx 10^\circ$.

has a finite width and what is recorded is the cross-section of this with the Ewald sphere. As the Ewald sphere cuts the arc at an angle θ , a further factor of $\cos\theta$ is present. The corrected intensity is given by:

$$I_{real} = I_{ob} \frac{4\pi}{\lambda} \sin\theta \cos\theta \Delta \quad (2.4)$$

I_{real} is the corrected intensity, I_{ob} is the observed intensity and Δ is the estimated mosaicity; see figure-2.5 for an explanation of how Δ is estimated.

While applying geometrical corrections to the observed intensities we have to take into account the fact that the sample is **unoriented** in two dimensions and oriented in the third direction; that is, lamellae are oriented parallel to the glass surface but the

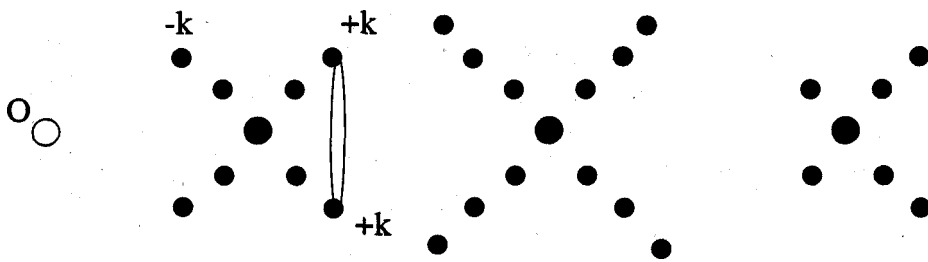


Figure 2.6: **Schematic diagram of a typical diffraction pattern from the ripple phase.** The open circle marked 'O' corresponds to the origin. The big filled circles are the main reflections and the small filled circles are the satellite reflections. The $+k$ and $-k$ reflections are marked. Each satellite is a ring which cuts the Ewald sphere twice thus giving rise to two spots.

ripple direction is not fixed. We assume that the ripple orientation is uniformly distributed, that is, all orientations are equally possible. For the lamellar reflections, the previous discussion holds and the corrected intensities can be calculated using eq.-2.4.

Since the direction of the ripple is not fixed, the satellite reflections form a ring around each lamellar reflection (fig. 2.6). The observed spots are where these rings intersect the Ewald sphere, each ring thus gives rise to two equivalent spots. Because of the smearing effect discussed before, the cross-section of the ring broadens. This ring intersects the Ewald sphere at a small angle and so the total integrated intensity of the spot has a very weak angular dependence (proportional to cosine of the angle), provided it is cut twice and not once only at its edge. Thus the correction due to the mosaic spread is automatically taken care of. But this is only a cross-section and so has to be multiplied by the perimeter of the ring to get the total intensity. The radius of this ring is $\frac{2\pi|k|}{\lambda_r} \sin\gamma$ where k is the corresponding Miller index. Therefore for the satellites,

$$I_{real} = I_{ob} \frac{4\pi^2}{\lambda_r} |k| \sin\gamma \quad (2.5)$$

where, I_{real} is the corrected intensity and I_{ob} is the observed intensity. The numerical factors have to be taken care of properly since unlike in a fully oriented or fully unoriented sample, the numerical factors for lamellar and satellite reflections are different.

2.3.3 Absorption corrections

As the x-rays travel through the sample, they get absorbed and thus lose some of their intensity. This happens both before and after scattering. In the present geometry, unlike in conventional powder samples, different parts of the samples diffract into different orders (figure-2.2). Therefore the path traveled inside the medium, and hence the absorption correction, is different for each order. For this reason, absorption corrections may be quite important here.

To calculate the absorption, it is assumed that the glass surface on which the sample is deposited, is opaque to x-rays. The path traveled (before and after scattering) by x-rays diffracted from each part of the sample is calculated from geometrical considerations. The absorption coefficient (μ) is estimated (from the values of the atomic absorption coefficients given in standard tables and using the volumetric data of Wiener et. al. [6]), to be equal to 9/unit length. The absorption corrected intensity is given by:

$$I_{abcorr} = I / \int_{l=0}^{\delta} e^{-\mu p(l)} dl \quad (2.6)$$

where $p(l)$ is the path traveled by the x-rays inside the medium (see figure-2.7 for the expression for $p(l)$). Here δ is the thickness of the sample, R is the radius of the beaker on which the sample is deposited, θ is the Bragg angle for that reflection.

Since the thickness of the sample is difficult to measure, we have not been able to take absorption corrections into account. To check if this omission seriously affects our final electron density maps, we have assumed some reasonable values for the thickness (of the order of 10 - 100 microns) and applied the corrections. We find that the final electron density map is not affected much by these corrections. Therefore we are justified in neglecting absorption In the calculations presented here for the oriented samples.

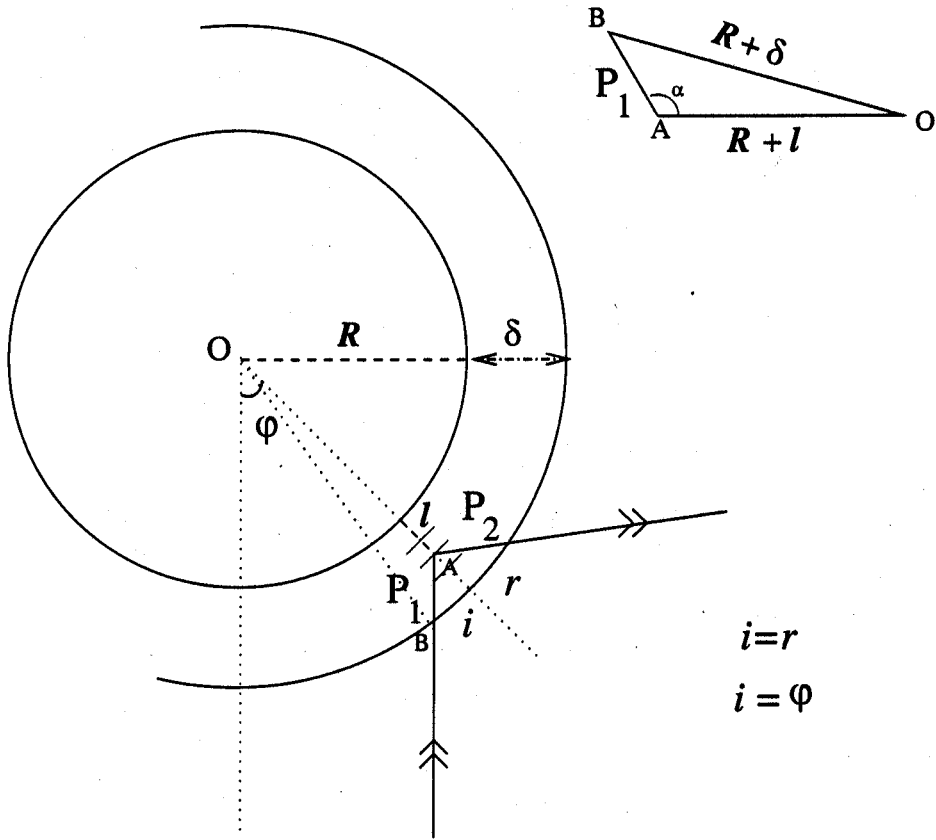


Figure 2.7: Calculation of the path traveled by x-rays inside the sample. The inner circle of radius R is the beaker, the sample is of thickness l . P_1 and P_2 are the paths traveled by the x-rays (marked by double arrow) before and after reflection from the sample. To calculate P_1 , the triangle OAB is considered. From geometrical considerations, the Bragg angle $\theta = \pi/2 - \phi$. Also, angle $\alpha = \pi - \phi$. Therefore, applying the rules of trigonometry to triangle OAB , P_1 is given by $-(R + l)\sin(\theta) + [(R + \delta)^2 - (R + l)\cos^2(\theta)]^{1/2}$. Since the rays are Bragg reflected, from the laws of reflection and geometry it can be shown that, $P_2 = P_1$. Therefore, $p(l) = 2P_1(l)$.

2.4 Modeling of the ripples

As discussed before, only the intensities ($|F_{\mathbf{q}}|^2$) and not the structure factors ($F_{\mathbf{q}}$) themselves are recorded in a x-ray diffraction experiment. The major task is therefore to find the phase of each reflection. We have adopted a modeling and least squares fitting procedure first used by Sun et. al. [7] in the context of the $P_{\beta'}$ phase. In this approach, a function representing the electron density in one unit cell of the $P_{\beta'}$ phase (in real space) is first constructed such that this function incorporates known and plausible features of the structure. This function has free parameters built into it to account for the imprecisely known features. This trial function is Fourier transformed to get the trial diffraction pattern (eq.-2.1). The values of these parameters are determined by a standard least squares fitting procedure where the calculated structure factors are fitted to the observed structure factors. The Fourier components corresponding to the final fitted parameters yield the phases of the reflections. These phases are now combined with the observed intensities and inverse Fourier transformed to give the final electron density map (eq.-2.1). The electron density $\rho(x, z)$ at a point (x, z) is given by:

$$\rho(x, z) = \sum_h \sum_k \Phi_c^{hk} |F_o^{hk}| \cos(q_x^{hk}x + q_z^{hk}z)$$

where Φ_c^{hk} is the phase calculated from the fit and $|F_o^{hk}|$ the observed structure factor for a spot with Miller indices (h, k) . A schematic diagram representing the above procedure is given in figure-2.8. The modeling step is crucial since a wrong or bad model may not lead to the correct structure. It is important to look at the final electron density map and accept it only if there are no features that are known to be obviously incorrect.

The electron density within the unit cell, $\rho(x, z)$, is described as the convolution of a ripple contour function $C(x, z)$ and the transbilayer electron density profile $T_{\psi}(x, z)$:

$$\rho(x, z) = C(x, z) * T_{\psi}(x, z)$$

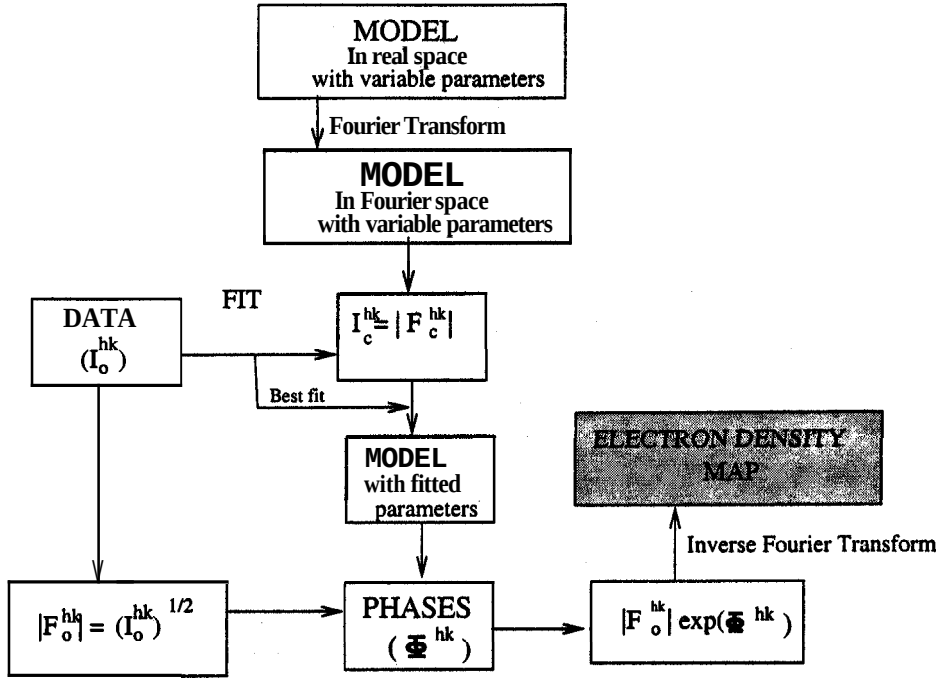


Figure 2.8: **A schematic diagram of the fitting procedure.**

The ripple contour function is written as $C(x, z) = \delta(z - u(x))$, where $u(x)$ describes the ripple profile. In reciprocal space the convolution becomes a simple multiplication. The structure factor $F(\mathbf{q})$ is therefore given by

$$F(\mathbf{q}) = F_C(\mathbf{q})F_T(\mathbf{q})$$

where $F_C(\mathbf{q})$ and $F_T(\mathbf{q})$ are the Fourier transforms of $C(x, z)$ and $T_\psi(x, z)$ respectively.

2.4.1 Specific forms of $C(x, z)$ and $T_\psi(x, z)$

Ripple shape: $C(x, z)$

The ripple shape is taken to have the form of a saw-tooth with **peak-to-peak** amplitude A , λ_1 is the projection of the longer arm of the saw-tooth on the x-axis (figure-2.9).

This is exactly same as the shape considered in ref. [7]. The origin of the unit cell is chosen such that the ripple profile is explicitly centrosymmetric. Then the shape

function $u(x)$ is given by:

$$u(x) = \begin{cases} -\frac{A_r}{\lambda_r - \lambda_1}(x + \frac{\lambda_r}{2}) & \text{if } -\frac{\lambda_r}{2} \leq x < -\frac{\lambda_1}{2} \\ \frac{A_r}{\lambda_1}x & \text{if } -\frac{\lambda_1}{2} \leq x \leq \frac{\lambda_1}{2} \\ -\frac{A_r}{\lambda_r - \lambda_1}(x - \frac{\lambda_r}{2}) & \text{if } \frac{\lambda_1}{2} < x \leq \frac{\lambda_r}{2} \end{cases}$$

Therefore,

$$F_C(\mathbf{q}) = \frac{1}{\lambda_r} \int_{-\frac{\lambda_r}{2}}^{\frac{\lambda_r}{2}} dx dz e^{i[q_x x + q_z z]} \delta(z - u(x)) \quad (2.7)$$

$$= \frac{1}{\lambda_r} \int_{-\frac{\lambda_r}{2}}^{\frac{\lambda_r}{2}} dx e^{i[q_x x + q_z u(x)]} \quad (2.8)$$

$$= \frac{\lambda_1}{\lambda_r} \text{sinc}(\omega) + \frac{\lambda_r - \lambda_1}{\lambda_r} \frac{\cos \frac{1}{2}(\frac{1}{2}q_x \lambda_r + \omega)}{\cos \frac{1}{2}(\frac{1}{2}q_x \lambda_r - \omega)} \\ \times \text{sinc}(\frac{q_x \lambda_r}{2} - \omega)$$

where $\text{sinc}(\omega) \equiv \frac{\sin(\omega)}{\omega}$ and

$$\omega = \frac{1}{2}(q_x \lambda_1 + q_z A_r)$$

q_x and q_z are given in eq.-2.3 in terms of the Miller indices and the lattice parameters.

λ_1 and A_r are the adjustable parameters.

A different $C(x, z)$

A different, more smooth initial model was also tried for $C(x, z)$ to check if the final shape is an artifact of the specific initial model. Here

$$u(x) = a \sin(\frac{2\pi x}{\lambda_r}) + b \sin(\frac{4\pi x}{\lambda_r}) \quad (2.9)$$

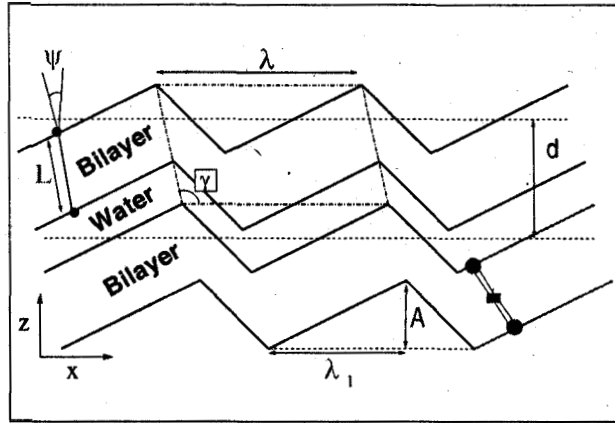


Figure 2.9: Schematic showing the structural parameters of the ripple phase.

a and b are the adjustable parameters.

Trans-bilayer profile ($T_\psi(x, z)$)

$T_\psi(x, z)$ gives the electron density at any point (x, z) in the bilayer along a straight line, which makes an angle ψ with the z-axis. The electron density in the methylene region of the bilayer is close to that of water and is taken as zero. We have used three models for $T_\psi(x, z)$, two of which are equivalent to the **SDF** and **M1G** models of ref. [7]. (model I and II below respectively).

Model I: In model I, $T_\psi(x, z)$ is taken as consisting of two delta functions with positive coefficients ρ_H , corresponding to the head group regions separated by a distance L , and a central delta function with negative coefficient of magnitude ρ_M , corresponding to the methyl region. The variable head-to-head distance L accounts for both chain melting and a tilt in the direction perpendicular to the rippling direction. This can be represented by

$$T_\psi(x, z) = \delta(x + z \tan \psi) \left\{ \rho_H \left[\delta\left(z - \frac{L}{2} \cos \psi\right) + \delta\left(z + \frac{L}{2} \cos \psi\right) \right] - \rho_M \delta(z) \right\} \quad (2.10)$$

Taking Fourier transform,

$$F_T(\mathbf{q}) = \rho_m \left\{ (\rho_H / \rho_M) \cos\left(q_z \frac{L}{2} \cos \psi - q_x \frac{L}{2} \sin \psi\right) - 1 \right\} \quad (2.11)$$

The adjustable parameters here are: ψ , $\frac{\rho_H}{\rho_M}$ and L (see figure-2.9).

Model II: The head groups and the chains can be more realistically modeled by replacing the delta functions representing the head groups and chains by Gaussians of width σ_h and σ_m , respectively. The electron density in the minor arm is allowed to be different by a factor f_1 from that in the major arm. The region where the two arms meet is modeled as a wall with an electron density differing by a factor f_2 from the rest of the arm. The wall thickness in this model is fixed at a value that is small compared to the bilayers thickness, half of the wall region being in each arm. The final form factor $F(\mathbf{q})$ reads:

$$F(\mathbf{q}) = F_C^M \cdot F_T^M + f_1 F_C^m \cdot F_T^m + f_2 (F_C^M \cdot F_T^M + f_1 F_C^m \cdot F_T^m) \quad (2.12)$$

where F_C^M and F_C^m are the contributions to F_C from the major and minor arms respectively and F_T^M and F_T^m are the respective contributions to F_T . The prime indicates that the contribution comes from the wall region. The Fourier transform of the transbilayer profile for the major arm is:

$$F_T^M = \frac{\rho_{H1}}{\rho_{M1}} e^{-\sigma_{h1}^2 \frac{(q_z - q_x \tan \psi_1)^2}{2}} \cos\left(q_z \frac{L_1}{2} \cos \psi_1 - q_x \frac{L_1}{2} \sin \psi_1\right) - e^{-\sigma_{m1}^2 \frac{(q_z - q_x \tan \psi_1)^2}{2}} \quad (2.13)$$

and for the minor arm is:

$$F_T^m = \frac{\rho_{H2}}{\rho_{M2}} e^{-\sigma_{h2}^2 \frac{(q_z - q_x \tan \psi_2)^2}{2}} \cos\left(q_z \frac{L_2}{2} \cos \psi_2 - q_x \frac{L_2}{2} \sin \psi_2\right) - e^{-\sigma_{m2}^2 \frac{(q_z - q_x \tan \psi_2)^2}{2}} \quad (2.14)$$

The labels 1 and 2 refer to the major and minor arm, respectively. In this model the following conditions are imposed:

$$L_1 = L_2, \sigma_{h1} = \sigma_{h2}, \sigma_{m1} = \sigma_{m2}, \frac{\rho_{H1}}{\rho_{M1}} = \frac{\rho_{H2}}{\rho_{M2}} \text{ and } \psi_1 = \psi_2 \quad (2.15)$$

The shape function $u(x)$ is the same as eq.-2.7 but now the calculation of the Fourier transforms for the minor and major arm and also the wall region (eq.-2.8) has to be done separately. There are 10 adjustable parameters in this model.

Model III: In the models I and II, the head-to-head distance L is fixed along a direction making an angle ψ w.r.t the z -axis. This forces bilayer thicknesses along the layer normal to be different in the two arms. Therefore in model III, the restrictions imposed upon the models I and II were lifted, so that the length L of the chains in the two arms need not be the same. The parameters L , ψ , σ_h, σ_m and ρ_H/ρ_M were allowed to be different in the two arms of the ripple.

$$L_1 \neq L_2, \sigma_{h1} \neq \sigma_{h2}, \sigma_{m1} \neq \sigma_{m2}, \frac{\rho_{H1}}{\rho_{M1}} \neq \frac{\rho_{H2}}{\rho_{M2}} \text{ and } \psi_1 \neq \psi_2 \quad (2.16)$$

The expressions for F_T^M and F_T^m are same as in equations-2.13 and -2.14 except that now the equalities-2.15 are not imposed. Further, the wall between the two arms was taken to have a variable width w . This model has 15 adjustable parameters. Minimization is done by iterative least squares fitting with respect to six variables at a time [8].

2.5 The fitting procedure

The adjustable parameters (henceforth referred to as a_i s) in the models discussed above are determined by a non-linear least squares fitting procedure that uses the Lavenberg-Marquardt Method [8]. In this method some initial value is chosen for each a_i . The $F(\mathbf{q})$ corresponding to each observed reflection is calculated. Let the estimated $F(\mathbf{q})$ for a spot with Miller indices (h, k) be F_e^{hk} . Let the square-roots of the observed intensities with the same Miller indices be $|F_o^{hk}|$. Then the deviation is given by:

$$\Sigma = \sum_{h,k} || F_e^{hk} | - | F_o^{hk} ||^2$$

This quantity is minimized using the conjugate gradient method [8]. In this method, not only the function to be minimized (C) but also the derivatives of this function with respect to all the parameters ($\frac{\partial \Sigma}{\partial a_i}$) have to be supplied. The derivatives were determined analytically with the help of the mathematical software package 'Maple'. The final converged values of the a_i s are put back into the expression for F_e^{hk} to get the best-fit structure factors F_f^{hk} . The phase Φ_c^{hk} of the spot with Miller indices (h, k) is $\frac{F_f^{hk}}{|F_f^{hk}|} = \pm 1$ (since the structure is centro-symmetric). The product of F_o^{hk} and Φ_c^{hk} is calculated to give the final set of $F(\mathbf{q})$ s which is then inverse Fourier transformed to get the electron density maps.

2.6 Conclusion

In this chapter we have discussed the details of our experimental and data reduction procedure. We have shown that absorption corrections do not significantly change the calculated electron density profiles. We are unable to analyze the data from oriented samples using models II and III, due to the lack of convergence resulting from the large number of adjustable parameters in these models. The shape function given by eq.-2.9 also leads to a similar lack of convergence in case of oriented samples. Hence all the data from oriented samples were analyzed only with $T_\psi(x, z)$ given by model I and $C(x, z)$ given by a sawtooth shape: The electron density maps obtained using the procedure described in this chapter will be discussed in chapter-111.

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Chapter 3

Structure of the Asymmetric Ripple Phase

3.1 Introduction

In this chapter we present electron density maps of various lipids (namely, DMPC, POPC, DHPC and DLPC) in the $P_{\beta'}$ phase. In section-3.2 we discuss our motivation for the present study. In section-3.3 we discuss the structure of the ripple phase inferred from the electron density maps of DMPC. We argue that all the features of the electron density maps are compatible with a tilt of the chains along the rippling direction. In section-3.4, we discuss the ripple shape and the temperature dependence of ripple parameters in POPC, DHPC and DLPC. In section-3.5 we discuss the influence of chirality on the ripple profile and show that molecular chirality does not affect the shape of the ripples. Our results are summarized in section-3.6.

3.2 Motivation

Though some of the structural aspects of the ripple phase were well established [1, 2, 3, 4, 5, 6] the finer features were poorly understood when we started our studies on this phase. The ordering of the hydrocarbon chains within the bilayer is one such feature. Though as mentioned in chapter-I, the chains are mostly stiff and have some sort of positional order [2], diffusion and NMR experiments indicate that there is considerable amount of disorder associated with the chain region [7, 8]. The nature of this disorder is still a matter of debate. Empirically, only lipids that have a $L_{\beta'}$ phase

at lower temperatures exhibit the ripple phase ¹, indicating the importance of the tilt of the chains in the formation of the ripples [10].

Though it is clear from x-ray data that the chains are tilted in the $P_{\beta'}$ phase [2], the direction of this tilt is not known. The chain reflections recorded in experiments are rather broad and therefore it has not been possible to obtain the tilt of the chains in the ripple phase directly from x-ray data. This broadening of the chain reflections happens because of one or both of the following reasons: the presence of disordered chains can broaden the spots or there might be an inherent spread in the spots because of the fact that the chains are arranged on a rippled bilayer rather than on a flat bilayer.

Many of the theories of the ripple phase are based on models where the tilt of the chains play an important role. For example, the Chen-Lubensky-MacKintosh model [11, 12] (see chapter-V) forbids a tilt of the chains along the rippling direction, and the Seifert-Shillock-Nelson model [13] (see chapter-V) is based on the idea that the tilt in the two monolayers of the bilayer are decoupled. Therefore determining the direction of the chain tilt should provide some important clues to construct a theory of the ripple phase.

Recently, Sun et. al. [14], have calculated the electron density map of DMPC in the ripple phase from the x-ray data of Wack and Webb [15]. This map shows a saw-tooth shaped ripple profile. They find that: (a) the bilayer thickness in the smaller arm of the saw-tooth is smaller than that in the longer arm and is comparable to the bilayer thickness in the L_{α} phase. The bilayer thickness in the longer arm is found to be about the same as that in the $L_{\beta'}$ phase. (b) The electron density in the head-group region is similarly lower in the smaller arm. Based on the above observations they hypothesized that the chains in the longer arm **of** the ripple are

¹DHPC is an exception. At high hydrations and low temperatures, it has an interdigitated gel phase (L_{β_I}) rather than a tilted gel phase ($L_{\beta'}$) but at higher temperatures it has a $P_{\beta'}$ phase [9]

stretched like in the $L_{\beta'}$ phase, whereas the shorter arm has disordered L_{α} like chains.

However, the above scenario is incompatible with other experimental observations. One such example is the diffusion experiment of Schneider et. al. [8]. In these experiments, self-diffusion in the ripple phase is found to be highly anisotropic, with a fast component that is 4-5 orders of magnitude faster than the slow component; but the fast component itself is about 2-3 orders of magnitude smaller than that in the L_{α} phase. Thus the authors of Ref. [8] conclude that although the intramolecular hydrocarbon chain disorder may be substantial in the fast bands, the intermolecular order in this region is not like that in the L_{α} phase.

Another compelling evidence against the L_{α} - $L_{\beta'}$ micro-phase-separation picture is from calorimetric data (this argument was first discussed by Carlson and Sethna [16]). The ratio of the lengths of the two arms of the ripple, as observed in the electron density map is about 1:2. Therefore according to the micro-phase-separation picture, at the L_{α} to $P_{\beta'}$ phase transition (the main-transition), about $\frac{2}{3}$ of the chains become all-trans. The rest of the chains can go to the all-trans conformation either gradually thus giving rise to a temperature dependence of the ratio of the lengths of the two arms or abruptly at the $P_{\beta'}$ to $L_{\beta'}$ transition (the pre-transition). Our experiments (discussed later in this chapter) rule out a temperature dependence of the ratio of the two arms. Therefore $\frac{1}{3}$ of the chains should go from L_{α} like to $L_{\beta'}$ like configuration at the pre-transition. This should imply that the ratio of the latent heat of the main-transition to the latent heat of the pre-transition should be roughly 2:1. But the ratio observed is about 10:1. Thus the micro-phase-separation picture is incompatible with diffusion and calorimetric experiments.

One of the motivations for recalculating the electron density maps of DMPC was to see if the results of Sun et. al. [14] was an artifact of their initial model for the **trans**-bilayer electron density profile. In their model, by construction, the **bi**-

layer thickness is different in the two arms of the ripple. It is partly to account for this features that they propose the micro-phase-separation model. Therefore it was important to check the correctness of the electron density maps they obtained, before attempting to explain the maps in terms of an alternative structure of this phase.

Though for a long time it was clear from x-ray and freeze fracture data that the stable ripples seen in experiments are asymmetric (without a mirror plane normal to the rippling direction), the actual shape of the ripples was not well established. A recent freeze fracture experiment [17] showed that the ripples have a profile that can be described by

$$h(x) = a \cos(qx) + b \cos(2qx) \quad (3.1)$$

where $h(x)$ describes the ripple profile along the rippling direction $\hat{x}$, q is the wave-number and a and b are constants. The ripple shape was thus predicted to be rather smooth. On the other hand, the electron density map of Sun. et. al. exhibit a less smooth, saw-tooth like profile. But since this initial model for fitting the ripple profile was also a saw-tooth, checking the model independence of the shape of the ripple was another objective of our analysis.

In previous work, the deviation of the unit cell parameter y from $\frac{\pi}{2}$ (see chapter-II for the definition of y) was always taken to be a direct indication of the asymmetry of the ripple profile. We argue that this need not necessarily be correct. The word asymmetric (symmetric) is used to indicate the absence (presence) of a plane of reflection in a direction perpendicular to the ripple wave-vector. A distinction should be made between the symmetry of the ripple profile (by which we mean the height variation of the bilayer) and the symmetry of the rippled bilayer itself. For concreteness consider a saw-tooth/triangular profile: the arguments presented here hold for a more smooth (sinusoidal like) profile also. In previous work, $\gamma = \frac{\pi}{2}$ was assumed to imply ripples where the lengths of the two arms are equal (symmetric profile) and $\gamma \neq \frac{\pi}{2}$ was assumed to imply ripples where the two arms are of unequal length (asymmetric

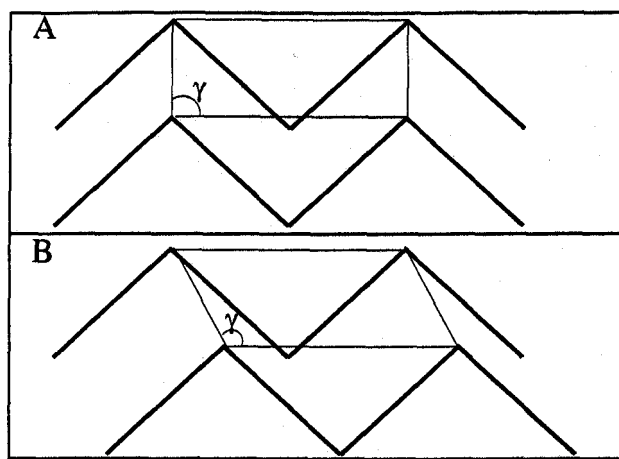


Figure 3.1: **A: The ripple profile is triangular with two equal arms and $\gamma = \frac{\pi}{2}$. B: The two arms of the ripple are still equal but now $\gamma \neq \frac{\pi}{2}$**

profile). But this assumption is valid only when the layer thickness in the two arms is the same (see figure-3.1). If the layer thickness is allowed to be different in the two arms, a symmetric profile (with two arms equal) is possible even when $\gamma \neq \frac{\pi}{2}$. Therefore, to ascertain the symmetry of the ripple profile, it is not enough to look at γ ; it is necessary to get the actual shape of the profile.

In the absence of complete structural information, a theory of the ripple phase that explains all the experimental observations is difficult to formulate. Lubensky and MacKintosh [11, 12] proposed a Landau theory that predicts the formation of an asymmetric ripple phase in chiral bilayers. In this theory, the tilt of the hydrocarbon chains is the order parameter and a coupling between the divergence of the tilt and the mean membrane curvature can give rise to a height modulation of the bilayer. In the case of achiral bilayers, this model predicts the existence of two symmetric ripple phases in addition to a square lattice phase. When the system is chiral, one of these two phases becomes asymmetric. Thus, according to this phenomenological model, achiral bilayers can exhibit only symmetric ripples, whereas both symmetric and asymmetric ripples can occur in chiral bilayers [11, 12] (see chapter-V for detailed discussions). In order to test these predictions concerning the influence of molecular chirality on the structure of the ripple phase, Katsaras and Raghunathan

[19] carried out x-ray diffraction experiments on aligned films of **chiral (l)** and racemic (dl) dimyristoylphosphatidylcholine (DMPC) bilayers.

The experiments were done on a racemic mixture rather than a pure achiral compound because of the following reason. An organic molecule is chiral if it has **atleast** one carbon atom which is attached to four different groups. From figure-1.1 (**chapter-I**) it is clear that the carbon atom in **2** position in the backbone of a DMPC molecule has four different groups attached to it and hence DMPC is chiral. The achiral version of this molecule is 1,3 DMPC where the chains are attached to carbons 1 and 3 and the head-group is attached to carbon **2**. This compound does not have a ripple phase (and incidentally has a L_{β_I} phase rather than a $L_{\beta'}$ phase at lower temperatures). Therefore in order to investigate the influence of chirality, it is necessary to look at racemic mixtures.

Under similar experimental conditions, the diffraction patterns from the chiral and racemic DMPC in the $P_{\beta'}$ phase were practically indistinguishable. Moreover, both the systems were found to have an oblique unit cell, which was taken as an indication of the presence of asymmetric ripples.

The observation of asymmetric ripples in the **racemate** can still be reconciled with the predictions of the **Lubensky-MacKintosh** model if the d and l enantiomers within each layer phase separate into chiral domains. To investigate this possibility, Katsaras et. al. performed calorimetric measurements on mixtures of l-dipalmitoylphosphatidylcholine having perdeuterated hydrocarbon chains (**l-DPPC-d₆₂**) and d-DPPC [20]. The main-transition in these mixtures is analogous to liquid to solid transitions. The calorimetric behavior of such mixtures can be any one of the following:

- **A** mechanical mixture or conglomerate: such mixtures exhibit one eutectic point. The mixture in the solid **phasé** ($P_{\beta'}$ in this case) consists of crystallites of the two enantiomers.

- Racemic compound: molecules of the *d* and the *l* enantiomers get closely associated to form a compound. Such mixtures exhibit two eutectic points.
- Solid solution: the mixture is homogeneous and the two enantiomers are present unordered in the solid phase. The phase diagram has no eutectic point. The melting point gradually changes as the concentration is varied.

The data for *l*-DPPC-*d*₆₂ and *d*-DPPC is consistent with a binary system whose components exhibit complete mutual solid solubility. This suggests that the asymmetric ripples seen in racemic DMPC multibilayers are not the result of the formation of chiral domains due to separation of the two enantiomers.

As mentioned in chapter-11, the wavelength of the ripples (λ_r), the bilayer periodicity (*d*) and the angle (*y*) between the two axes of the unit cell are structural features that can be obtained directly from a diffraction pattern of oriented samples. The values of these three parameters are found to be comparable in chiral and racemic DMPC bilayers under similar experimental conditions. But as explained before, it is necessary to look at the detailed structure of the ripples to ascertain the shape of the ripple profiles. In order to check whether the detailed shape of the ripples in these two systems are also similar, we have calculated the electron density profiles of the ripple phase of *l* and *dl*-DMPC.

3.3 Electron density map of DMPC

3.3.1 The model

The data from Ref. [15] was fitted using three different models for $T_\psi(x, z)$ (see chapter-11). For powder samples of DMPC [15], models I, II and III give the same phases except for the faint (0,*k*) reflections². Model III gives only a marginally better fit than model II. The values of *L* (*L* is the layer thickness along a direction making

²the phases are different for the faint reflection (3,0) also.

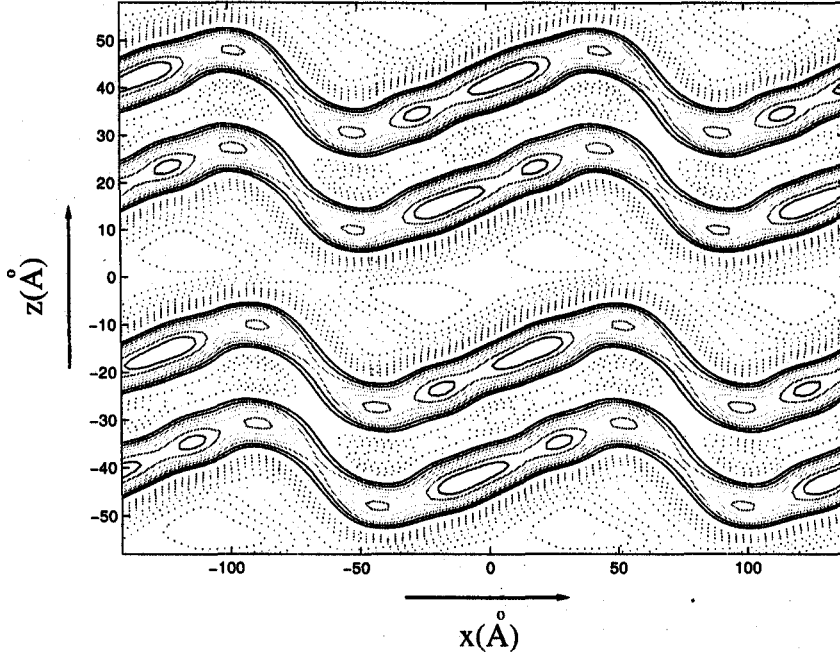


Figure 3.2: Electron Density Map of the ripple phase of DMPC. Temperature = 18.2°C and partial specific volume of water = 0.263 (Data from [15]). The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 40 Å, whereas that of the water region is about 15 Å.

an angle ψ with the x-axis : see chapter-I1 for details) in the two arms are almost the same and comparable to that obtained from model II. This is also true of the other parameters which are allowed to be different in the two arms. However, model III gives a slightly higher value of 0.7 for f_1 (f_1 is the ratio of the electron density in the shorter arm to that in the longer arm). As discussed below, this factor can be accounted for in terms of the chain tilt, without resorting to the assumption of a L_a -like organization in the minor arm. The low Σ ($=\sum_{h,k} ||F_e^{hk}| - |F_o^{hk}||^2$) and R ($=\frac{\sum_{h,k} ||F_e^{hk}| - |F_o^{hk}||}{\sum_{h,k} |F_o^{hk}|}$) values for models II and III, and the absence of any physically unacceptable features in the electron density map (figure-3.2) indicate that these models closely represent the true structure of the system. The observed structure factors and those calculated using the three different models are given in table-3.1 and the converged values for the model parameters are given in table-3.2.

Table 3.1: **Observed $[|F_o(h,k)|]$ and calculated $[F_c(h,k)]$ structure factors obtained using the three models:** DMPC powder data at 18.2°C and partial specific volume of water = 0.263. Data from [15].

h	k	$ F_o(h,k) $	$F_c(h,k)$		
			Model I	Model II	Model III
0	1	5.3	-1.7	9.2	8.5
0	2	9.7	1.2	-5.8	-3.1
0	3	7.8	-0.7	1.9	-2.2
1	-1	60.8	-99.3	-60.2	-59.3
1	0	100.0	-49.0	-99.7	-100.9
1	1	26.9	41.6	26.5	26.2
1	3	7.6	17.4	2.9	5.4
2	-2	15.1	-71.6	-12.5	-16.2
2	-1	71.2	-34.1	-71.4	-70.1
2	0	39.7	-11.6	-43.3	-44.1
2	1	33.9	29.2	31.7	31.3
2	2	22.7	-24.8	-22.7	-21.5
2	3	14.2	19.2	14.2	14.1
2	4	7.8	-13.2	-7.3	-9.6
3	-2	29.3	43.6	27.6	29.0
3	-1	44.2	28.1	45.7	44.3
3	0	12.0	-3.0	2.9	3.8
3	2	10.5	12.7	14.4	14.2
3	3	14.9	-14.8	-13.1	-12.5
3	4	10.0	14.0	9.6	9.8

Table 3.2: **The values of the structural parameters obtained from the three models.** DMPC powder data at 18.2°C and partial specific volume of water = 0.263. 2D-oblique lattice parameters are: $\lambda_r=141.7 \text{ \AA}$, $d=58.6 \text{ \AA}$ and $\gamma=98.4^\circ$. (Data from [15].)

Parameter	Model I	Model II	Model III
$A_r(\text{\AA})$	19.7	18.7	19.1
$\lambda_1(\text{\AA})$	106.2	101.0	101.5
ψ	5.7"	9.9"	9.1°
$L(\text{\AA})$	41.0	37.0	37.2,37.0*
ρ_h/ρ_m	1.1	0.9	1.1,1.1*
f_1	-	0.6	0.7
f_2	-	0.9	0.65
σ_h	-	4.9	4.6,4.7*
σ_m	-	13.3	9.4,10.0*
$w(\text{\AA})$	-	2.0(fixed)	9.2
Σ	692.5	217.6	213.6
R	0.172	0.083	0.087

* indicates the values in the shorter arm

3.3.2 Existence of average tilt of the chains along the ripple

The electron density map of the ripple phase of **DMPC**, calculated with the data of [15] is shown in figure-3.2. The ripples clearly have a saw-tooth shape, with an offset between the two leaves of the bilayer. The simplest explanation for this offset is an average tilt of the chains along the rippling direction; such an offset cannot be expected if the tilt were in a plane normal to the rippling direction. The tilt angle ψ is found to be approximately equal to $(\gamma - \frac{\pi}{2})$. Further confirmation of the existence of an average tilt along this direction comes from the fact that the value of L (the layer thickness along ψ) is almost equal in the two arms and is comparable to twice the length of a fully stretched DMPC molecule (37.6 Å [21]). If it is assumed that the chains are tilted at an angle ψ with the z -axis, their tilt with respect to the local layer normal can be calculated from the shape of the ripple. Using the values of the structural parameters given in table-3.2, the tilt angle with respect to the local layer normal turns out to be 1.6° and 34.5' in the longer and shorter arms, respectively. The tilt in the short arm is comparable to that found in the $L_{\beta'}$ phase. Since the area per molecule is inversely proportional to the cosine of this angle, a value of 0.82 is obtained for f_1 . This is in very good agreement with the value of 0.77 obtained from the map for the ratio of the average electron densities in the head-group region of the longer and shorter arms. Thus an average tilt of the chains along the rippling direction provides a consistent explanation for many features of the electron density map. This means that to a good approximation the height modulation of the bilayers along the x -axis can be described as arising from a relative sliding movement of neighboring chains, with all the chains lying the x - z plane and tilted by a constant angle ψ with respect to the z -axis (see figure-3.3). The existence of an average chain tilt along the rippling direction breaks the reflection symmetry of the bilayer in the plane normal to it and hence can be expected to be responsible for the asymmetric ripples seen in this system.

We have confirmed that even if the initial model for the ripple profile is taken to

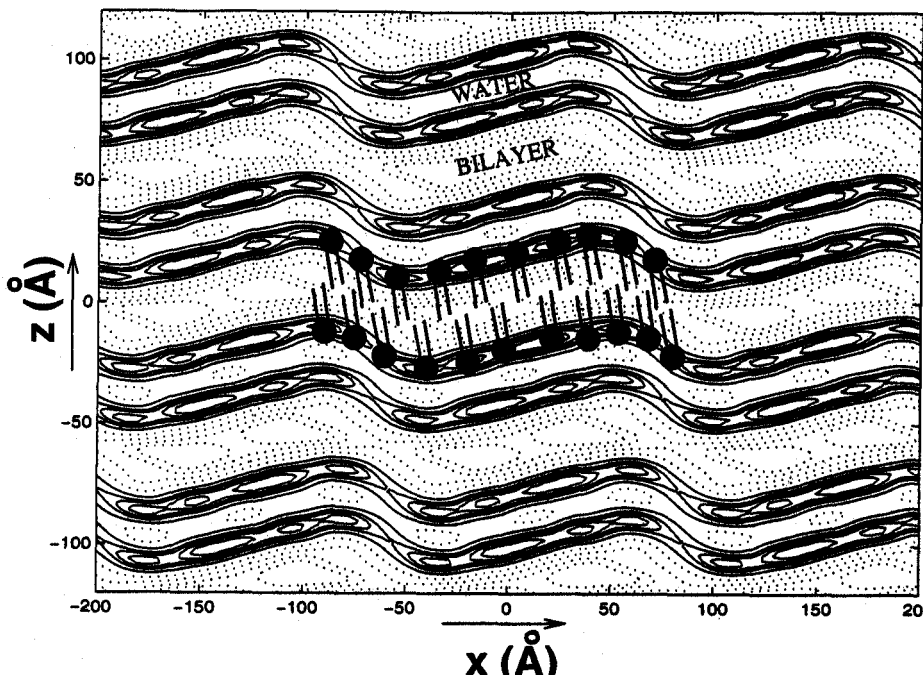


Figure 3.3: **The arrangement of the lipid molecules in the ripple phase.** The electron density map is same as in figure 3.2

be a more smooth sinusoidal-like shape (eq.-2.9), the final electron density map is still saw-tooth like. We find that the phases are insensitive to the precise details of initial shape chosen for $u(x)$. This indicates that the saw-tooth shape obtained for the ripple profile is not an artifact of the initial model. But if the initial shape is very different from the actual shape, for example if $u(x)$ is chosen to be a simple sinusoidal function, then the fit is very bad and the final electron density map has a lot of obviously unphysical features like discontinuity of bilayers.

3.3.3 Temperature dependence

We have also calculated the electron density maps of DMPC at different temperatures in the ripple phase, using data from oriented films. The structural features of the ripples are found to be similar to those obtained from the data of Ref.- [15]. The temperature dependence of the structural parameters of the ripples in DMPC are found to be very weak (table-3.3), as in the case of DPPC [18]. Contrary to results of freeze fracture experiments [17], we find that in all the cases studied, the ripple shape has Fourier components higher than the second. Further, we do not

Table 3.3: **The structural parameters for DMPC at different temperatures.**

T (°C)	λ_r (Å)	d (Å)	γ (°)	λ_1 (Å)	A_r (Å)
25	140±2	55.6±0.1	99±1	90±1	19±1
24	142	55.8	99	91	20
23	142	55.9	100	88	19
22	144	56.0	100	87	19
21	145	56.3	99	95	20
20	154	56.4	99	98	20

find a significant temperature dependence of the amplitude of the ripples, again in contrast to the results of ref.- [17]. The electron density map of DMPC at different temperatures is given in figures-3.4 and 3.5. The observed and calculated structure factors corresponding to the figures is given in table-3.4 and the converged values of the fitted parameters are given in table-3.5. The values of Σ and R for these fits are about 3500 and 0.3 respectively.

3.4 Electron density maps of POPC, DHPC and DLPC

3.4.1 POPC: structural features and temperature dependence

The head-group and the linkage group in POPC is the same as that in DMPC but the chains have 18 and 16 carbon each and the 18 carbons long chain has a double bond. Thus POPC differs considerably from DMPC in the chain region. The electron density map of the ripple phase of POPC at different temperatures and 75% RH is shown in figure-3.6. This data-set is from oriented samples. The converged values of the fitted model parameters are given in table-3.6 and the observed and calculated structure factors corresponding to the figure are given in table-3.7. For these fits, the values of Σ and R are about 1000 and 0.3 respectively. The ripple shape is very similar to that of DMPC. Like DMPC, POPC ripples also have a saw-tooth shape and an offset between the monolayers indicating an average tilt in the direction of rippling. In POPC, the angle γ is much **larger** than in DMPC, whereas the wavelength and layer spacing are comparable. Unlike those of DMPC, the structural features of

Table 3.4: The observed and calculated structure factors for oriented sample of DMPC at different temperatures.

		25°C		24°C		23°C		22°C		21°C		20°C	
h	k	$ F_o $	F_c	$ F_o $	F_c	$ F_o $	F_c	$ F_o $	F_c	$ F_o $	F_c	$ F_o $	F_c
1	0	100.0	-85.4	100.0	-80.2	100.0	-77.8	100.0	-78.0	100.0	-79.3	100.0	-84.8
1	-1	44.8	-39.8	42.5	-38.0	43.0	-37.3	-	-	48.6	-40.4	49.4	-40.7
1	1	-	-	-	-	-	-	42.2	38.0	-	-	-	-
2	0	31.3	-20.9	29.7	-19.8	28.8	-23.1	26.9	-21.5	28.9	-20.1	28.1	-21.6
2	-1	50.2	-42.1	43.6	-40.5	42.5	-38.2	41.6	-38.8	49.2	-43.4	49.4	-47.8
2	-2	10.2	-15.9	9.3	-15.4	9.5	-14.1	9.1	-16.6	12.5	-16.1	15.1	-20.7
2	2	17.5	-14.6	15.7	-14.6	15.9	-22.2	14.9	-15.6	17.1	-19.3	16.4	-16.6
2	3	13.7	7.7	10.6	7.9	10.9	13.3	8.3	7.5	11.1	12.6	13.0	8.4
3	0	24.1	-2.6	21.2	-2.9	21.0	-1.8	20.4	-1.7	23.6	-5.6	25.3	-4.8
3	-1	46.4	34.9	42.4	33.9	40.2	31.7	37.5	33.6	42.2	38.6	42.5	36.8
3	-2	30.6	32.5	29.2	32.0	28.4	28.9	27.6	32.4	31.7	37.8	33.6	38.2
3	2	10.0	17.1	9.5	17.1	-	-	22.3	21.2	30.2	20.9	-	-
3	3	18.0	-15.4	16.5	-15.9	27.6	-21.0	13.5	-17.9	15.2	-22.4	12.3	-18.3
3	4	17.2	9.7	16.3	10.3	17.8	13.3	14.1	9.7	15.7	17.4	15.9	11.4
3	5	-	-	12.4	-4.3	10.7	-4.4	12.3	-2.3	13.8	-9.4	12.9	-4.2
3	6	-	-	-	-	-	-	-	-	14.5	2.3	-	-
4	0	32.1	29.5	29.9	27.9	29.0	25.9	28.0	26.3	32.0	27.7	33.6	29.5
4	-1	30.1	-34.5	25.5	-31.2	23.3	-30.2	21.6	-29.0	22.7	-26.5	25.5	-26.0
4	-2	59.1	-84.3	55.5	-79.5	56.2	-75.6	52.5	-71.5	59.9	-81.4	60.1	-79.9
4	-3	29.4	-35.1	27.7	-33.9	26.9	-36.9	27.1	-35.9	31.4	-37.2	33.2	-40.4
5	0	10.6	0.3	8.5	0.6	7.7	3.0	6.8	1.6	6.9	1.7	5.9	1.7
5	-1	6.9	0.6	5.9	0.9	7.3	1.3	-	-	4.9	2.2	6.1	2.8
5	-2	-	-	7.8	-10.1	8.7	-10.6	8.5	-11.7	8.8	-12.8	8.7	-16.6
5	-3	-	-	-	-	8.7	-12.0	10.2	-17.6	10.2	-17.8	-	-
6	0	4.9	0.9	4.4	1.2	4.2	1.1	3.3	0.9	3.7	3.4	3.4	2.6
6	-1	5.9	-4.9	5.9	-5.4	4.7	-6.4	-	-	-	-	5.3	-7.9
6	-2	-	-	-	-	-	-	8.2	7.1	-	-	-	-
6	-3	-	-	-	-	9.9	21.8	-	-	-	-	-	-
7	0	-	-	-	-	2.4	-16.9	-	-	3.5	-17.5	-	-
9	0	-	-	-	-	2.1	-0.1	-	-	-	-	3.1	0.3

Table 3.5: Converged values of the fitted parameters for DMPC at different temperatures.

	25°C	24°C	23°C	22°C	21°C	20°C
$\lambda_1(\text{\AA})$	89.8	91.3	87.8	87.5	94.9	97.6
$A_r(\text{\AA})$	19.4	19.6	19.2	19.2	20.5	20.3
ψ	5.7°	5.7°	11.4°	5.7°	5.7°	2.8°
ρ_H/ρ_M	1.4	1.5	1.4	1.5	1.6	1.5
$L(\text{\AA})$	40.2	40.2	40.2	40.2	40.2	40.2

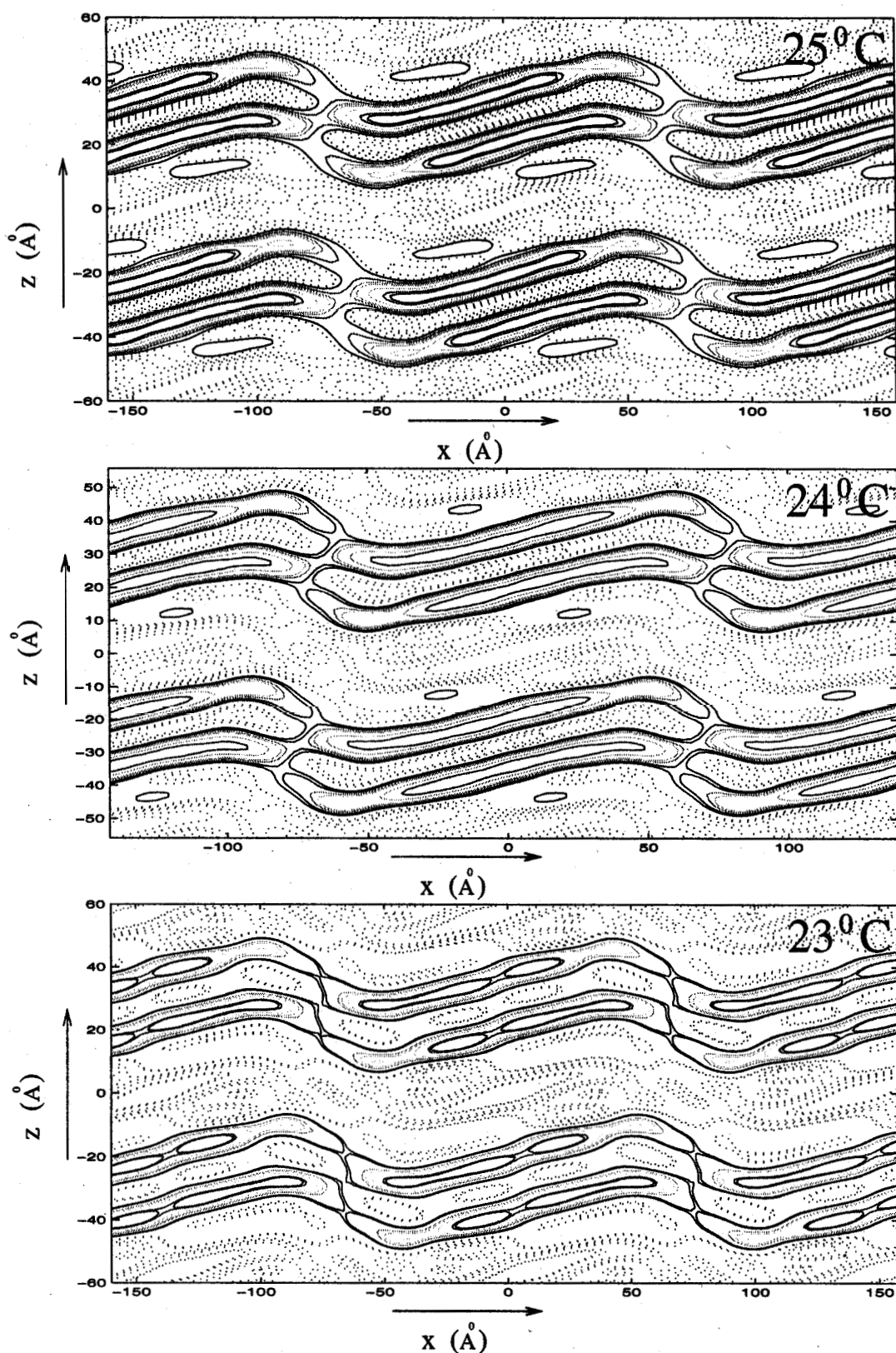


Figure 3.4: Electron Density Map of the ripple phase of 1-DMPC at different temperatures The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 40 Å, whereas that of the water region is about 15 Å.

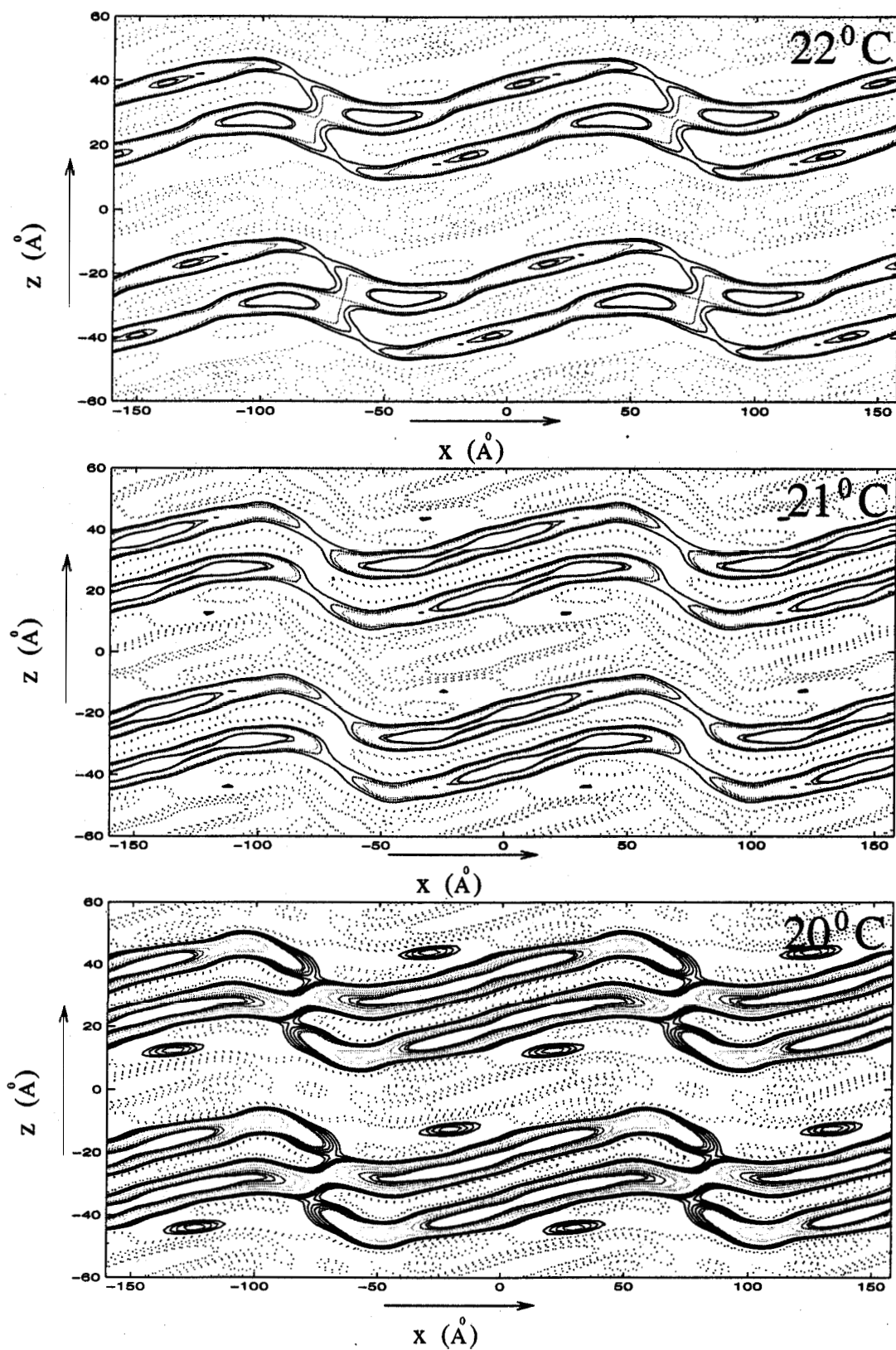


Figure 3.5: Continuation of figure 3.4.

Table 3.6: Converged values of the Atted parameters for the ripple phase of POPC at 75%RH.

T (°C)	$\lambda_1(\text{\AA})$	$A_r(\text{\AA})$	ψ	ρ_H/ρ_M	L(Å)
13.0	169.0	15.9	0.0°	1.3	38.8
13.5	127.3	13.9	0.5°	1.5	50.4
14.0	121.7	12.0	0.6°	1.4	51.2

the ripple phase of POPC vary significantly with temperature, as shown in table-3.8. The fits are not as good as in the case of DMPC data of ref. [15]. Hence the values of the last two parameters quoted in the table are the ones estimated from the electron density maps. The layer spacing decreases slowly and γ increases steadily as temperature is increased. The ripple wavelength first decreases and then suddenly increases to a large value just below the transition. These trends are very similar to those seen in DPPC [18], but in POPC the temperature dependence is very much pronounced. The amplitude, except near the L_α transition, is about 10 Å, half that in DMPC.

The double bond in one of the chains of POPC is known to lead to a kink in the chain when it goes to the fully stretched conformation. This results in lower main transition temperatures of such lipids. It is interesting that this feature does not seem to affect the structure of the P_β' phase. It is likely that as proposed by Cevc [22], the terminal region of the chain after the double bond is always in a molten conformation.

3.4.2 Oriented sample of DHPC

The main difference between DMPC and DHPC is that in DHPC the chains are connected to the backbone through ether rather than ester linkages. The crystallographic parameters at 37° C and 95% RH are: $\lambda_r = 140.9 \text{ \AA}$, $d = 62.3 \text{ \AA}$ and $\gamma = 100^\circ$. The temperature dependence of these parameters is negligible. The electron density map is given in figure-3.7. Though the map is not as good as the one obtained for DMPC, it can be seen that the general shape is again a saw-tooth with the two arms unequal. The map is consistent with a tilt of the chains along the rippling direction. The

Table 3.7: The observed and calculated structure factors for POPC oriented sample at different temperatures.

		13°C		13.5°C		14°C		14.5°C		15°C	
h	k	$ F_o $	F_c	$ F_o $	F_c	$ F_o $	F_c	$ F_o $	F_c	$ F_o $	F_c
1	0	100.0	-56.7	100.0	-100.6	100.0	-89.3	100.0	-95.8	100.0	-53.9
1	-1	28.5	-12.5	42.9	-27.9	29.8	-20.8	23.4	-6.9	7.9	0.7
1	1	26.4	21.3	39.8	29.8	27.6	22.2	20.7	22.7	8.5	-0.5
2	0	18.1	-38.1	22.0	-25.8	23.0	-30.8	22.7	-28.6	16.4	-92.0
2	-1	7.7	-52.8	11.6	-19.1	6.7	-7.3	-	-	3.2	3.5
3	0	11.6	8.9	14.9	14.2	19.1	17.9	21.0	18.2	15.9	19.1
3	-1	19.6	31.5	29.4	28.3	22.6	21.9	18.2	29.1	3.9	-0.3
3	1	7.0	-7.2	10.6	-13.7	14.2	-14.0	14.3	6.5	4.6	-0.8
3	2	10.8	5.2	16.3	13.7	16.4	13.0	9.4	-15.7	-	-
4	0	10.3	5.2	13.6	-6.2	36.1	-24.9	58.5	-58.8	35.1	5.3
4	-1	70.5	-32.9	106.1	-107.2	87.0	-95.9	77.9	-79.0	11.5	2.3
4	-2	28.1	-5.3	42.2	-41.9	26.8	-19.0	14.1	7.7	23.5	-0.8
4	1	19.8	1.1	29.8	24.2	28.2	29.9	26.8	26.3	10.6	-0.4
4	2	16.4	-6.1	24.6	-33.7	14.5	-31.9	9.0	-11.7	4.5	0.0
5	0	7.0	14.4	9.3	-5.3	9.5	-0.5	7.5	7.9	4.8	-0.3
5	-1	9.4	-50.3	14.1	22.7	11.8	16.2	13.7	-14.4	-	-
6	0	7.0	-7.5	-	-	-	-	7.0	6.6	4.8	5.9
6	-1	9.3	10.0	-	-	13.6	-11.8	17.0	12.2	-	-
6	-2	16.8	20.5	-	-	13.1	-14.8	12.6	3.9	-	-

Table 3.8: Temperature variation of the structural parameters of the ripple phase of POPC at 75%RH. From the electron density maps.

T(°C)	d(Å)	γ°	λ_r (Å)	A_r (Å)	$\frac{\lambda_l}{(\lambda_r - \lambda_l)}$
13.0	58.3±0.1	116±1	200±2	10±1	2.0±0.1
13.5	58.0	119	170	9	1.8
14.0	58.0	120	159	9.0	1.9
14.5	57.3	124	143	5	2.6
15.0	56.4	133	266	5	1.9

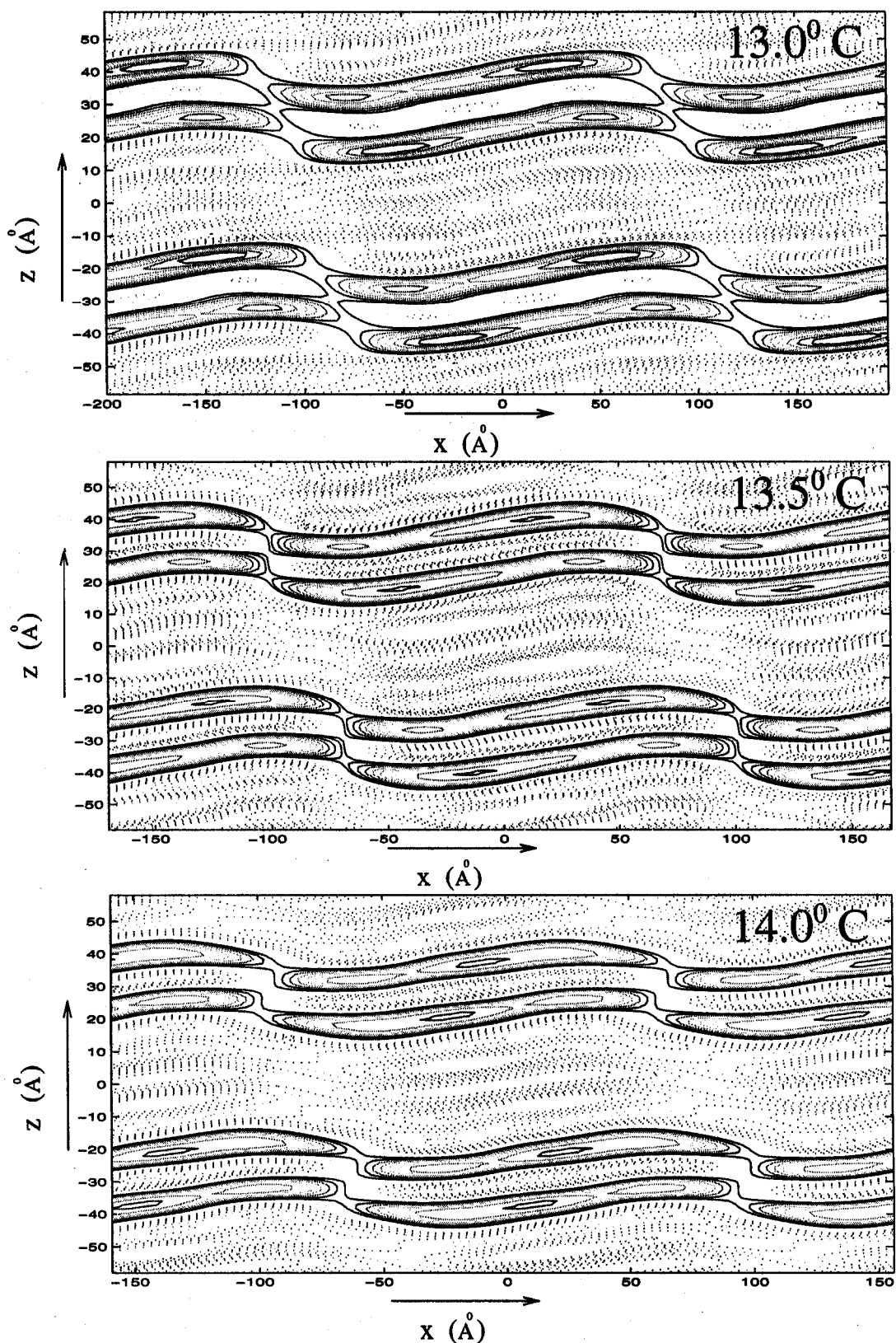


Figure 3.6: Electron density map of POPC at different temperatures. The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 40 Å, whereas that of the water region is about 15 Å.

Table 3.9: **The observed and calculated structure factors for oriented sample at of DHPC at 37° C and 95f2% RH.**

h	k	$ F_o $	F_c	h	k	$ F_o $	F_c
1	0	100.0	-50.8	3	-3	2.6	-0.6
1	-1	18.1	-24.8	3	3	2.6	-8.4
1	1	12.4	19.5	3	-4	4.2	-0.3
1	-2	6.6	1.5	3	4	4.2	6.9
1	2	6.6	-10.2	4	0	26.3	15.6
1	-3	5.7	1.7	4	-1	8.1	-31.0
1	3	5.7	4.5	4	1	4.7	-0.8
2	0	10.8	-14.7	4	-2	44.2	-52.2
2	-1	16.2	-20.1	4	2	4.7	-12.6
2	1	9.1	15.4	4	-3	5.7	-10.8
2	-2	4.7	-3.8	4	3	22.1	21.2
2	2	4.7	-14.4	4	-4	4.2	8.4
2	-3	5.7	1.8	4	4	4.2	-23.0
2	3	3.6	11.1	5	0	1.3	0.9
3	0	14.1	0.3	5	-1	2.1	-0.1
3	-1	13.5	11.4	5	1	2.1	-1.4
3	1	3.3	-5.0	6	0	1.4	0.0
3	-2	17.9	6.4	7	0	1.5	-8.7
3	2	3.6	7.8				

Table 3.10: **The converged values of the fitted parameter for DHPC**

$\lambda_1(\text{\AA})$	$A_r(\text{\AA})$	ψ	ρ_H/ρ_M	$L(\text{\AA})$
96.2	20.5	9.6°	1.0	46.2

observed and calculated structure factors are given in table-3.9 and the converged values of the fitted parameters are given in table 3.10. The value of Σ for this fit is about 5000 and the R-factor is 0.6. In all the compounds discussed so far that is, DMPC, POPC and DHPC, the ratio of the projected lengths of the major and minor arms is about 2 and is essentially insensitive to temperature. This observation further supports the view that the chain organization in the minor arm is not like that in the L_α phase.

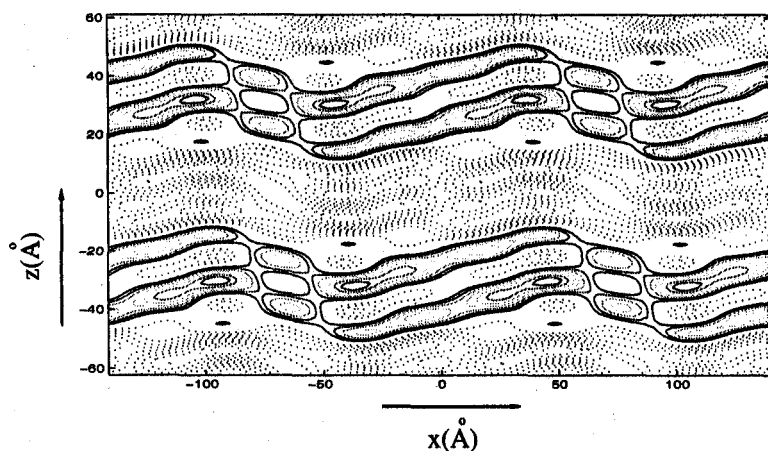


Figure 3.7: **Electron density map of DHPC at 37°C and $\approx 97\%$ RH.** The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 40 Å, whereas that of the water region is about 15 Å.

Mixture of DHPC and DPPC

The phase diagram of mixtures of DHPC and DPPC has been studied by Lohner et. al. [23]. These mixtures exhibit the $P_{\beta'}$ phase over a larger temperature range than the pure systems; the range being maximum ($\approx 20^\circ$) for the 1:1 mixture. Since this seems to be largest temperature range of the $P_{\beta'}$ phase, we have studied the temperature dependence of the ripple parameters in the 1:1 (wt.%) mixture. There were too few reflections, probably due to inherent disorder in the system, to do the kind of analysis that we have done for the pure samples. However, the crystallographic parameters can still be computed and are given in table-3.11. The values of the ripple parameters for the mixture are close to the values observed for the pure systems. The layer spacing (**d**) and the angle γ are insensitive to variations in temperature, whereas the wavelength decreases from a large value near the pre-transition to a value comparable to that in DPPC at higher temperatures.

3.4.3 Unoriented sample of DLPC

DLPC is similar to DMPC except that the chains are shorter (**12** carbons). We have phased the data of Tardieu et. al. [1] from unoriented sample of DLPC at -7°C and containing 77% by weight of water. The ripple parameters are: $\lambda_r=85.3\text{\AA}$, $d=$

Table 3.11: **The temperature dependence of the ripple parameters in 1:1 (wt%) mixture of DHPC and DPPC**

T(°C)	d(Å)	γ°	$\lambda_r(\text{Å})$
26	60 ± 2	94 ± 2	175 ± 5
30	60	94	157
34	62	98	147
38	62	99	151
42	58	95	132

Table 3.12: **The observed structure factors (F_o), the corresponding phases reported in ref. [1] (Φ_{rep}) and the phases calculated by us (Φ_{cal}).**

h	k	F_o	Φ_{rep}	Φ_{cal}	h	k	F_o	Φ_{rep}	Φ_{cal}
0	1	5.5	-1	-1	2	1	19.1	-1	+1
1	0	36.4	-1	-1	2	2	6.9	+1	-1
1	-1	10	+1	-1	3	-1	16.3	-1	+1
0	2	3.2	+1	+1	3	-2	10	+1	+1
1	1	5.5	-1	+1	3	0	5.7	+1	+1
1	-2	3.9	-1	-1	3	1	9.7	+1	-1
2	-1	22.4	+1	-1	3	2	8.3	+1	+1
1	2	3.0	+1	-1	4	-1	9.6	+1	-1
2	0	30	-1	-1	4	0	7.6	-1	-1
2	-2	21	-1	-1					

51.98, and $\gamma=110^\circ$. Figure-1.10 (chapter-I) shows the electron density map calculated using the phases originally assigned by Tardieu et. al. and figure-3.8 shows the map calculated using our modeling and fitting approach (the corresponding phases are given in table-3.12). The model parameters are given in table-3.13. For this fit, the value of Σ is about 100 and the R-factor is about 0.2. The new set of phases leads to a more uniform thickness of the head-group region and is thus probably closer to the correct set. The bilayers in this case have a triangular shape. The major and the minor arms are more or less of equal length. But the ripple profile still lacks a mirror plane normal to the rippling direction because the thickness of the two arms are not equal. The electron density in the head-group region is again higher in the thicker arm and the value of the model parameter L is comparable to twice the length of a fully stretched DLPC molecule (32.6 Å). Thus the structure of this system is also consistent with the presence of an average chain tilt along the rippling direction.

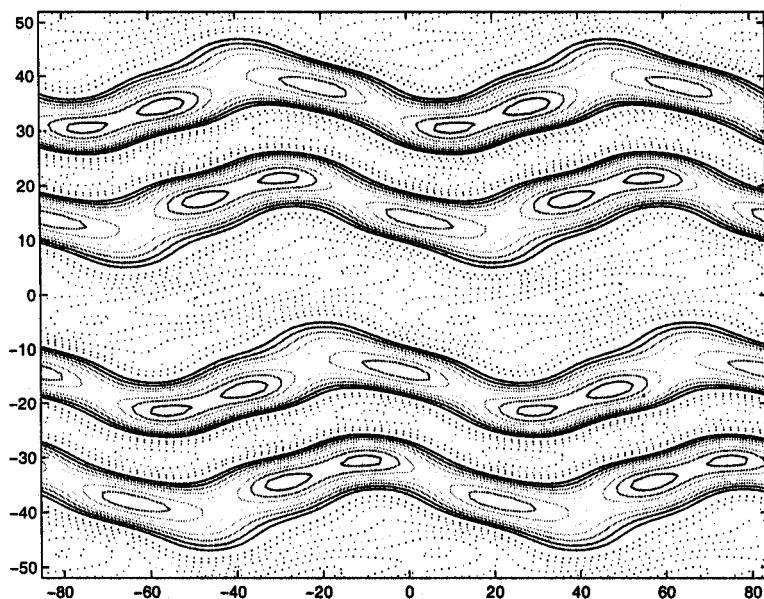


Figure 3.8: Electron density map of DLPC at -7°C and 77% by weight of water, using the phases calculated by the modeling and fitting procedure. Data from ref.- [1]

4

Table 3.13: The converged values of the fitted parameter for DLPC

$\lambda_1(\text{\AA})$	$A_r(\text{\AA})$	ψ	ρ_H/ρ_M	$L(\text{\AA})$
39.4	11.5	18.2 °	1.2	34.6

3.5 Influence of chirality

To check the influence of chirality on the ripple phase, the electron density maps of 1 and dl-DMPC under similar experimental conditions have been calculated. The observed and calculated structure factors for 1 and dl-DMPC at different temperatures and $98\pm 2\%$ RH are given in table-3.14. Almost all the phases of the corresponding reflections in the two cases are the same. The electron density profiles at 24°C for 1 and dl-DMPC are presented in figures 3.9 and 3.10. The structural parameters of the ripple phase at two different temperatures are given in table-3.15. As one can observe, the ripples formed by chiral and racemic DMPC bilayers do not differ appreciably. The peak-to-peak amplitude in both cases is approximately $18\text{ \AA}$, and the ratio of the length of the major arm to that of the minor arm is found to be 2.1 and 2.4 for 1-DMPC and dl-DMPC bilayers, respectively. Furthermore, the thickness of the bilayer in the two arms is different in both cases. This can be explained, as discussed before, in terms of an average tilt of the hydrocarbon chains along the ripple wave-vector. Thus at all temperatures studied, the structure of the ripple phase in chiral and racemic DMPC bilayers is practically identical.

3.6 Conclusion

We have calculated the electron density maps of the ripple phase of DMPC, POPC, DHPC and DLPC. The shape of the ripples in these systems are very similar and all of them exhibit asymmetric ripples that lack a plane of reflection normal to the ripple wave-vector. We do not see any temperature dependence of the shape of the ripples though the values of the structural parameters do vary with temperature. The structural parameters of DMPC has a very weak temperature dependence whereas in case of POPC the dependence is rather strong.

From our analysis, we have been able to, establish the existence of an average chain tilt in the direction of rippling, which is probably responsible for the asymmetric

Table 3.14: The observed and calculated structure factors at 24°C and 98% RH of chiral and racemic DMPC.

		l-DMPC		dl-DMPC	
h	k	$ F_o $	F_c	$ F_o $	F_c
1	0	100.0	-80.2	100.0	-117.1
1	-1	42.5	-38.0	59.2	-32.9
2	0	29.7	-19.8	65.0	-43.3
2	-1	43.6	-40.5	68.0	-55.2
2	-2	9.3	-15.4	29.0	-38.5
2	2	15.7	-14.6	21.7	-3.2
2	3	10.6	7.9	10.6	0.3
3	0	21.2	-2.9	32.9	11.5
3	-1	42.4	33.9	53.3	39.3
3	-2	29.2	32.0	35.0	38.3
3	2	9.5	17.1	9.0	16.0
3	3	16.5	-15.9	12.3	-0.5
3	4	16.3	10.3	13.3	2.0
3	5	12.4	-4.2	-	-
4	0	29.9	27.9	31.1	22.5
4	-1	25.5	-31.2	33.1	-37.4
4	-2	55.5	-79.5	56.8	-57.5
4	-3	27.7	-33.9	27.9	-35.8
5	0	8.5	0.6	9.6	4.6
5	-1	5.9	0.8	12.0	-0.3
5	-2	7.8	-10.1	10.8	-29.4
6	0	4.4	1.2	6.4	-5.6
6	-1	5.9	-5.4	-	-
7	0	-	-	5.5	-2.2
9	0	-	-	4.5	-1.3

Table 3.15: The structural parameters of the ripple phase of chiral and racemic DMPC.

		$\gamma(^{\circ})$	$\lambda(\text{\AA})$	d	$\lambda_1(\text{\AA})$	$A_r(\text{\AA})$
l-DMPC	24 ° C	99±1	142±2	56±1	97±2	18±1
	21 ° C	99	145	56	98	18
dl-DMPC	24 ° C	98	141	56	100	19
	21 ° C	98	140	56	99	18

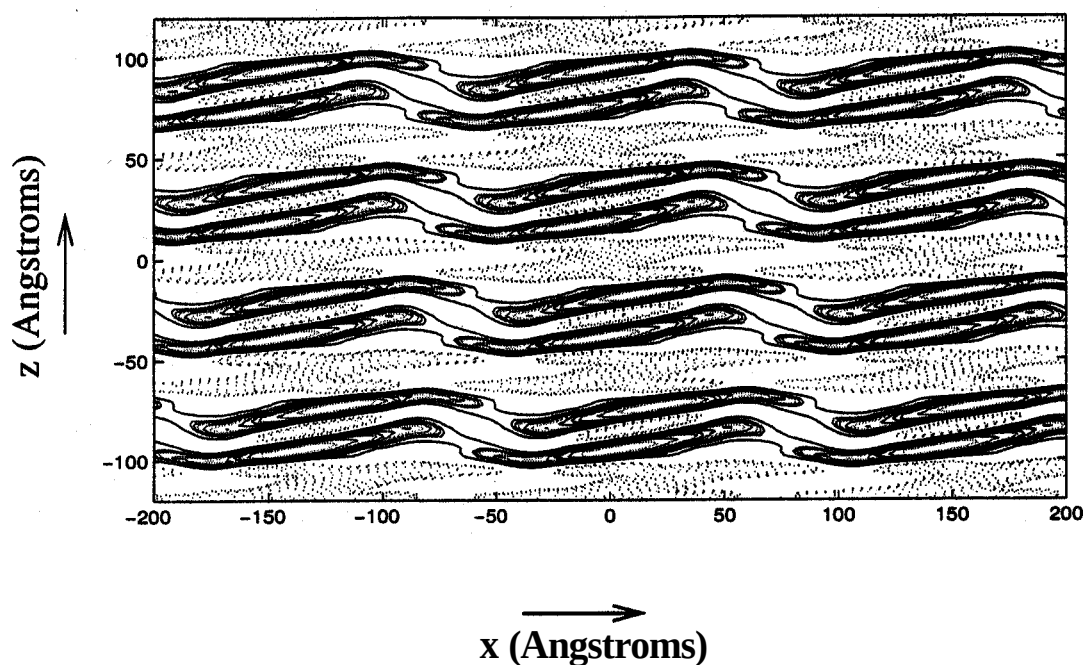


Figure 3.9: Electron Density Map of the ripple phase of dl-DMPC at 24°C and 98% RH. The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 40 Å, whereas that of the water region is about 15 Å.

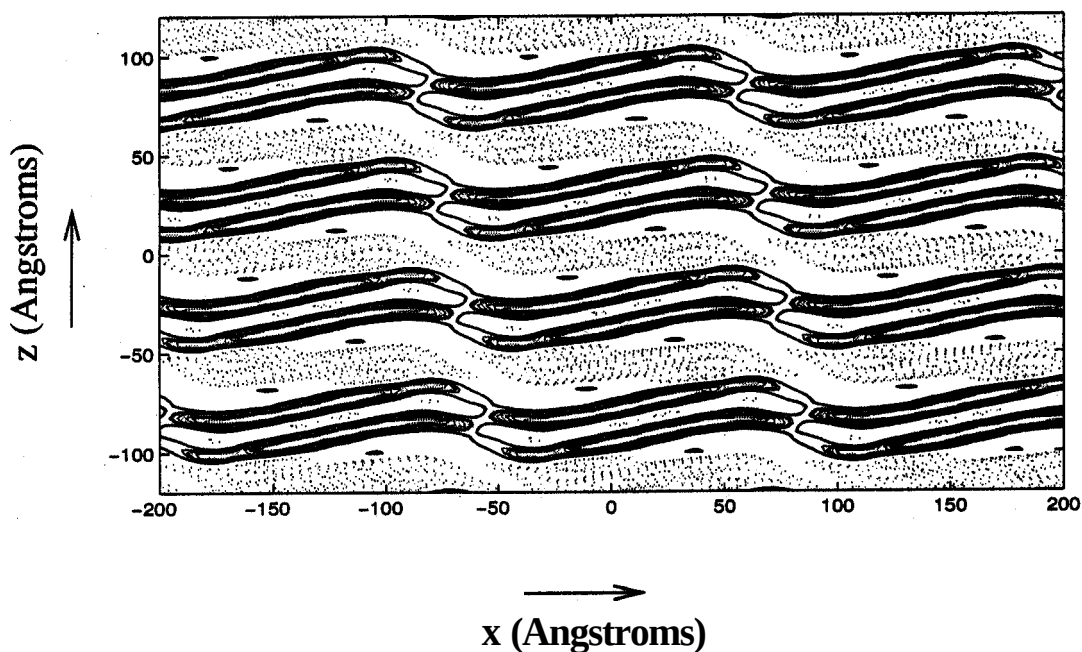


Figure 3.10: Electron Density Map of ripple phase of l-DMPC at 24°C and 98% RH.

ripples seen in these systems. Such a structure is supported by the electron density maps of all the systems studied. The height profile of the bilayers in DMPC, POPC and DHPC has a saw-tooth shape. The width of the shorter arm is comparable to the bilayer thickness. In DLPC the height profile is closer to triangular, with the width of the two arms almost equal and comparable to the bilayer thickness. It is interesting that the wavelength of the ripples in DLPC ($\approx 80 \text{ \AA}$) is the lowest reported in the literature. From our result it looks as if the minimum width of an arm of the ripple is set by the bilayer thickness. In that case, a value of about $80 \text{ \AA}$ may be the lowest possible for the wavelength of the ripples.

We have presented electron density profiles of chiral and racemic DMPC bilayers in the ripple phase. All the structural features in the two cases are found to be practically identical under similar experimental conditions. In particular, the ripples are asymmetric in both these systems. The present study thus unambiguously confirms that the structure of the $P_{\beta'}$ phase does not depend on the chirality of the lipid molecules constituting the bilayer.

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