Synopsis

This thesis deals with the structure of the ripple phase exhibited by some hydrated phospholipids. Two types of ripple phases have been seen in these systems. The most often seen is an asymmetric phase, where the lipid bilayers lack a plane of reflection normal to the rippling direction. The other is a metastable symmetric phase, where such a plane of reflection is present. We have calculated the electron density maps of the asymmetric phase of a few lipids from x-ray diffraction data. These maps show that in all the systems studied, the bilayers in this phase have a saw-tooth like height profile. Based on these maps we propose a new structural model for this phase, which is consistent with all the experimental observations. We have also calculated the electron density map of the metastable symmetric phase. Our results on this phase are in broad agreement with structural features obtained from earlier freeze-fracture electron microscopic studies.

It is clear from our studies that the asymmetry of the ripples in the stable phase arises from the presence of a non-zero mean tilt of the hydrocarbon chains of the lipid molecules along the rippling direction. However, such a structure is not predicted by earlier theories of this phase. We have constructed a Landau theory of this phase, which takes into account the possible anisotropy of the bending modulus of a bilayer with tilt order. We show that a lower bending modulus along the tilt direction can result from the packing of the chains in the bilayer. A structure similar to that deduced from the electron density maps is obtained from this theory.

In **chapter-I** we give a brief introduction to the phases exhibited by phospholipids and discuss in detail the previous work on the ripple ($P_{\beta'}$) phase.

Phospholipids are amphiphilic molecules with a hydrophilic head and one or more hydrophobic chains. At high hydration, depending on the temperature, they exhibit a variety of lamellar phases. One such phase is the ripple or $P_{\beta'}$ phase which is characterized by a one dimensional periodic height modulation. The x-ray diffraction pattern from this phase can be indexed on a two dimensional oblique lattice. The wavelength (λ_r) of the height modulation, the bilayer periodicity (d) and the oblique angle (γ) are the unit cell parameters. The ripple phase has been seen in phosphatidylcholines (PCs), phosphatidylglycerols (PGs) and phosphatidic acids (PAs), which differ from one another in the composition of the head-group. Most of the studies have been on PCs.

The common type of ripples detected in x-ray experiments have an oblique unit cell and are referred to as 'asymmetric'. X-ray and freeze fracture analysis indicated that these ripples lack a plane of reflection normal to the rippling direction. λ_r=136 Å, d=71 Å and γ= 95^o are typical values for DPPC (Dipalmitoylphosphatidylcholine) in this phase. Metastable ripples are sometimes formed in DPPC and DMPC (Dimyristoylphosphatidylcholine) while cooling the sample from the high temperature phase. These metastable ripples are characterized by a 2-D rectangular unit cell (γ=90^o) and are referred to as symmetric ripples. A wavelength of about 265 Å, bilayer periodicity of about 80 Å and $\gamma = 90^o$ have been reported for the metastable ripples in DPPC. Thus the wavelength of this kind of ripples is about 1.8 times that of the coexisting asymmetric ripples. The bilayer periodicity is also larger as compared to the coexisting asymmetric ripples. By careful analysis of the height profile using freeze fracture techniques, it has been shown that these ripples have a groove at the top which gives them a non-centrosymmetric "M" shape. Each repeating "M" unit of the the symmetric ripple can be pictured as two one-wavelength-long units of the asymmetric ripples joined with one of the units rotated by 180^o; such a structure has the configuration "major arm-minor arm-minor arm-major arm" with the minor arms joining up to form the groove at the top.

In **chapter-II** we describe the data collection, data reduction and the modeling and fitting procedure used to construct the electron density maps. In this chapter we also give a brief introduction to x-ray diffraction.

A locally built temperature and humidity controlled heater is used to collect the data from oriented samples of DHPC (Dihexadecyl phosphatidylcholine). By depositing the sample on a curved surface, we get rid of the problem of rotating the sample since the curvature of the orienting surface introduces artificially the mosaicity that is necessary in order to scan many Bragg spots simultaneously. Cu K_α ($\lambda = 1.54$ Å) radiation originating from a rotating anode x-ray generator and rendered monochromatic by a graphite monochromator is used to illuminate the sample. The data is collected by an image plate; locally written programs using packages like 'matlab' and 'pg-plot' are used to display the diffraction patterns and get the integrated intensities. The diffraction pattern is indexed on a 2-D oblique lattice. Since the sample is oriented, the lattice parameters λ_r, d and γ can be directly read off from the diffraction pattern. In an x-ray experiment, only the intensities and not the structure factors themselves are recorded. The major task is therefore to find the phase of each reflection. For centrosymmetric structures, the phase factor is ± 1 whereas for non-centrosymmetric structures there is no such restriction on the phase factor. We have adopted a modeling and least squares fitting procedure to get the phases. In this approach, the electron density $\rho(x, z)$ within the unit cell is described as the convolution of a ripple contour function $C(x, z)$ and the transbilayer electron density profile $T_\psi(x, z)$. Here $\hat{x}$ is the rippling direction and $\hat{z}$ the normal to the average bilayer plane. The ripple contour function is written as $C(x, z) = \delta(z - u(x))$, where $u(x)$ describes the ripple profile. We model the ripple profile as a saw-tooth. The transbilayer profile $T_\psi(x, z)$ gives the electron density at any point (x, z) along a straight line, which makes an angle ψ with the z-axis. We use three models (with varying degrees of refinements and different numbers of fitting parameters) for $T_\psi(x, z)$ but

the final electron density map obtained using these different models do not differ appreciably. The adjustable parameters in the models are determined by a non-linear least squares fitting procedure. Some initial value is chosen for each of the fitting parameters and the structure factor corresponding to each observed spot is calculated. This is compared with the observed structure factor and the deviation is minimized. The final converged values of the parameters are put back into the expression for the trial function to get the best-fit structure factors. The products of observed structure factors and the calculated phases are inverse Fourier transformed to get the electron density maps.

In **chapter-III** we present the electron density maps of the stable asymmetric ripple phase of a few lipids. We have analyzed data mainly from four sources: (1) data from unoriented sample of DMPC using a synchrotron source (available in the literature), (2) data from oriented samples of DMPC and POPC (Palmitoyl-oleoyl phosphatidylcholine) (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 (Dilauroylphosphatidylcholine, available in the literature). Other data available in the literature have too many overlapping reflections to be suitable for this kind of analysis.

A typical electron density map (EDM) for DMPC is given in figure-1. The lattice parameters are: λ_r=141.7 Å, d=58.6 Å and γ=98.4^o. The peak-to-peak amplitude of the ripple is 20 Å, the length of the major arm of the saw-tooth is 100 Å, ψ=10^o and bilayer thickness along the straight line which makes an angle ψ with the z-axis is 40 Å. In all the lipids studied, the ripple profile has the saw-tooth shape seen in figure-1. The long arm of the saw-tooth is about twice as long as the shorter arm. Along the local layer normal, the thickness of the long arm is more than the thickness of the short arm, but along the direction defined by ψ, the thickness of the two arms are the same. Also, the electron density in the head-group region of the long arm

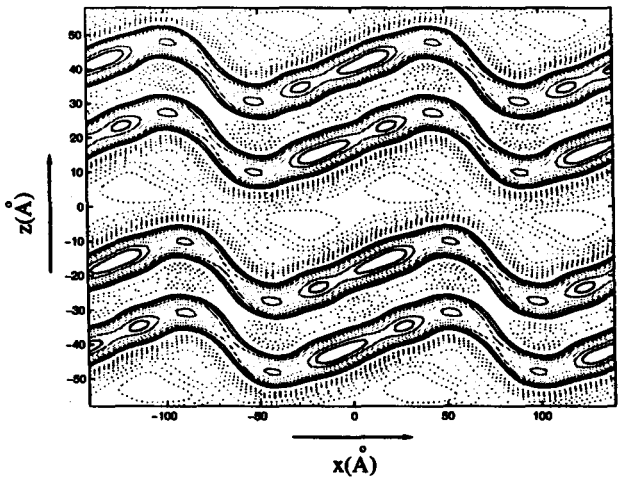

Figure 1: **Electron Density Map of the ripple phase of DMPC.** Temperature = 18.2°C and partial specific volume of water = 0.263. The positive (negative) contours are represented by solid (dotted) lines. The regions with positive electron density correspond to the head-groups. Note that the thickness of the bilayers is about 40 Å, whereas that of the water region is about 15 Å.

is larger than that of the shorter arm. These features of the EDM can be explained in terms of a tilt of the chains along the rippling direction by an angle of $(\gamma - \pi/2)$ with respect to the mean layer normal. The chain tilt with respect to the local layer normal in the longer arm is almost 0^o and that in the shorter arm is $\approx 30^o$. A careful inspection of the electron density map shows that the two monolayers of the bilayer are not in perfect registry and are shifted with respect to each other. This feature of the EDM can also be understood in terms of a chain tilt along the rippling direction.

It is found that for DMPC, the ripple parameters are more or less temperature independent. Similar analysis of POPC data reveals that though there is appreciable temperature dependence of the ripple parameters, the overall shape of the ripples remains the same. In view of theoretical predictions, we have investigated the influence of chirality on the structure of the ripple phase and have calculated the electron density maps of *l* and *dl*-DMPC under similar experimental conditions. At all temperatures studied, the structure of the ripple phase in chiral and racemic DMPC bilayers is found to be practically identical, indicating that chirality does not play any role in determining the symmetry of the ripples.

In **chapter-IV** we present the electron density maps of the metastable ripple phase calculated from x-ray data of oriented samples of DPPC. While the basic structural features of the stable asymmetric ripple have now been well established, even the shape of the metastable ripples is still a topic of debate.

We have calculated EDMs of the metastable ripples of DPPC at 39.2 °C and 100% relative humidity. The experiments were performed at the CHESS synchrotron facility (Cornell high-energy synchrotron source, Ithaca, USA) using incident radiation of wavelength 1.3808 Å. The details of data collection, data reduction and data analysis are same as in the case of the asymmetric ripples. From the diffraction patterns it can be seen that the angle γ is always $\pi/2$ for the metastable ripples. The lattice parameters for the metastable ripples at 39.2°C are: λ_r=254.5 Å, d=79.5 Å and γ=90°. To phase the reflections, different initial models for the ripple profile $C(x,z)$ have been tried, but none of them give a completely satisfactory electron density map. For the transbilayer profile $T_\psi(x,z)$, the simplest model was used.

Our electron density maps of the metastable ripples indicate that the ripple profile is most probably non-centrosymmetric but has a mirror plane perpendicular to the ripple wave-vector. The resolution of our EDMs is not good enough to make quantitative statements about the possible presence of the groove near the height modulation maxima that is seen in freeze fracture experiments. The amplitude of the metastable ripples is found to be about 50 Å. The thickness of the water layer is about 30 Å, about 10 Å more than in the asymmetric ripples. A bilayer thickness of about 50 Å indicates that the chains are mostly in the all-*trans* conformation. We also speculate on the origin of the difference in the water layer thickness in the two kinds of ripples.

A phenomenological theory of the ripple phase capable of accounting for asymmetric

ripples in achiral membranes is presented in **chapter-V**.

In this chapter we first describe the various theories that have been proposed so far for the ripple phase and point out the drawbacks of each theory. In particular, we discuss the Lubensky-MacKintosh model and then extend it by retaining some additional terms in the free energy density. In the Lubensky-MacKintosh (LM) theory, a coupling between the layer curvature and the gradient in the tilt gives rise to the modulated phase. The inter bilayer interactions are ignored; which is justified because ripples have been observed in unilamellar vesicles. For achiral systems, this theory predicts the existence of two symmetric ripple phases. They also predict a two dimensionally modulated square lattice phase. According to this theory, asymmetric ripples are allowed only in chiral systems. Thus this theory fails to explain the experimental observation of asymmetric ripples even in racemic mixtures. Further, the structure of the asymmetric phase (established in chapter-III) is not explained by this model.

In our theory (as in LM theory), the projection $\mathbf{m}$ of the molecular director onto the plane of the bilayer is the order parameter. $\mathbf{m}$ essentially has information about the tilt of the chains. The free energy density written in terms of $\mathbf{m}$ contains, apart from the usual Landau and elastic terms, a higher order gradient term and terms that allow for an anisotropy in the elasticity of the bilayers when there is a non-zero mean tilt. It is given by:

$$\begin{aligned} f &= \frac{1}{2}a|\,\mathbf{m}\,|^2 + \frac{1}{4}b|\,\mathbf{m}\,|^4 + \frac{1}{2}c(\nabla\cdot\mathbf{m})^2 + \frac{1}{2}\alpha(\nabla^2\mathbf{m})^2 + \frac{1}{4}\beta(\nabla\cdot\mathbf{m})^4 \\ &+ \frac{\xi}{2}[(\dot{\mathbf{m}}\cdot\nabla)\mathbf{m}]^2 + \frac{\zeta}{2}[(\mathbf{m}\times\nabla)\mathbf{m}]^2 \end{aligned} \tag{1}$$

In this theory, the following phases are obtained: (i) an L_α like phase with $\mathbf{m}$=0, (ii) a $L_{\beta'}$ like phase with $\mathbf{m} \neq 0$ and uniform everywhere, (iii) a symmetric ripple phase where $\mathbf{m}$ is spatially modulated around a zero mean (P_β), (iv) a symmetric ripple

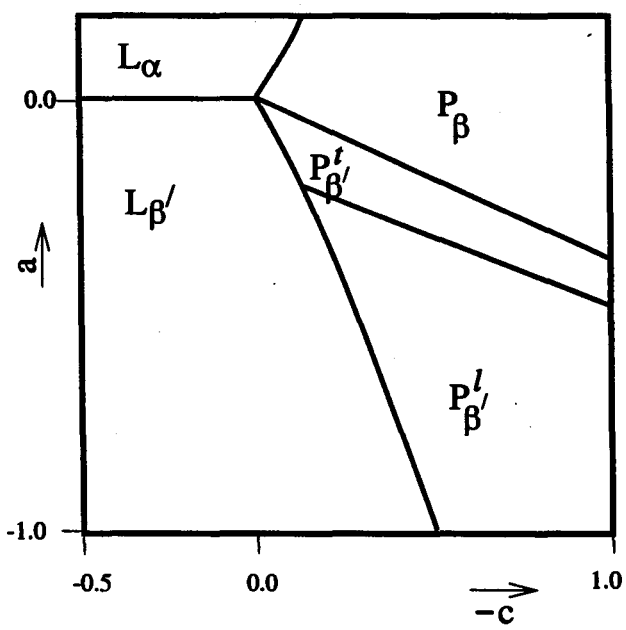

Figure 2: Numerically calculated mean-field phase diagram for an achiral membrane. The free energy density is given by Eq. 1 with b=1.0, α=0.01, β=4.0, ξ=0.1, ζ=0.7.

phase where **m** has a non-zero mean in a direction orthogonal to the modulation direction ($P^t_{\beta'}$) and (v) an asymmetric ripple phase, with the same symmetry as the experimentally seen $P_{\beta'}$ phase, where **m** is spatially modulated around a non-zero mean ($P^l_{\beta'}$). The phase diagram (for $\xi < \zeta$) is shown in figure-2. Thus our theory accounts for the structure of the ripple phase deduced from the electron density maps.

The following papers contain the work described in this thesis:

1. The structure of the ripple phase in chiral and racemic DMPC multibilayers,
   Kheya Sengupta, V. A. Raghunathan and John Katsaras,
   Phys. Rev. E **59** (2), 2455 (1999).
   cond-mat/9811370 (http://xxx.lanl.gov/)

2. Novel structural features of the ripple phase of phospholipids,
   Kheya Sengupta, V. A. Raghunathan and John Katsaras,
   Europhys. Lett. **49**, 722 (2000).
   cond-mat/9907016 (http://xxx.lanl.gov/)

3. Landau Theory of the ripple phase: asymmetric ripples in achiral systems,
   Kheya Sengupta, V. A. Raghunathan and Yashodhan Hatwalne,
   to be submitted.