Structure and phase behaviour of lipid–cholesterol membranes

by

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DECLARATION

I hereby declare that the work reported in this thesis is entirely original. This thesis is composed independently by me at Raman Research Institute under the supervision of Dr. V. A. Raghunathan. I further declare that the subject matter presented in this thesis has not previously formed the basis for the award of any degree, diploma, membership, associateship, fellowship or any other similar title of any university or institution.

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CERTIFICATE

This is to certify that the thesis entitled **Structure and phase behaviour of lipid–cholesterol membranes** submitted by Sanat Karmakar for the award of the degree of Doctor of Philosophy of Jawaharlal Nehru University is his original work. This has not been published or submitted to any other University for any other Degree or Diploma.

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Dedicated to
Ma and Baba

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Contents

Preface

This thesis deals with the structure and phase behaviour of lipid–cholesterol membranes. The distribution of cholesterol in biological membranes is known to be inhomogeneous. Cholesterol rich domains, called "rafts", have been proposed to exist in these membranes. Our motivation was to get some insight into the formation of these membrane rafts by studying model membranes containing cholesterol.

We have studied various phospholipid–cholesterol mixtures using x-ray diffraction and confocal fluorescence microscopy techniques. Oriented multilayers from these mixtures were used for x-ray diffraction, as they provide much more information, especially regarding the in-plane order of the bilayers, compared to unoriented samples. We have also studied some unoriented samples to examine the phase behaviour in excess water. One important result of these studies is the observation of a novel cholesterol induced modulated (P_β) phase in binary mixtures of cholesterol with dipalmitoyl phosphatidylcholine (DPPC) and dimyristoyl phosphatidylcholine (DMPC). Although the basic structural feature of the P_β phase is a height modulation of the bilayers, as in the ripple ($P_{\beta'}$) phase observed in between the main- and pre-transition in some phosphatidylcholines (PCs), these two phases are distinct. The dependence of the structural parameters on the cholesterol concentration (X_c) and on temperature in this phase shows opposite trend from that seen in the $P_{\beta'}$ phase. The electron density map of the P_β phase was calculated from the diffraction data. This phase has no average chain tilt with respect to the bilayer normal. In order to understand the role of chain tilt in the formation of the P_β phase, we have studied dilauryl phosphatidylethanolamine (DLPE)–cholesterol mixtures, as DLPE bilayers have no chain tilt in the gel phase. Interestingly, the P_β phase is absent in these mixtures. It is also not seen in unoriented samples of PC–cholesterol mixtures. However, they show a large d-spacing ($\sim$ 80 Å) in the cholesterol concentration range where the P_β phase is seen in oriented samples, indicating higher flexibility of the bilayers in this phase. Partial phase diagrams of these mixtures were deduced

from the diffraction data.

Giant unilamellar vesicles (GUVs) made from these binary lipid–cholesterol mixtures were also studied using fluorescence microscopy. We have observed the coexistence of gel and fluid phases at small values of X_c in GUVs made from DPPC–cholesterol mixtures. At these X_c, some GUVs exhibit significant thermal shape fluctuations, revealing an unexpected softening of the bilayer. However, GUVs made up of DMPC–cholesterol mixtures show non-spherical shapes, but do not exhibit significant thermal shape fluctuations. These results are in broad agreement with those obtained from the x-ray diffraction study.

Fluid–fluid immiscibility in ternary mixtures of cholesterol with a lipid containing saturated hydrocarbon chains, such as DPPC, and a lipid with unsaturated chains, such dioleoyl phosphatidylcholine (DOPC), is thought to be relevant for the formation of membrane rafts. We shall refer to these mixtures as ternary raft mixtures. Although fluorescence microscopy studies on GUVs show domains, indicating the coexistence of two fluid phases, none of the earlier diffraction studies have been able to detect it. However, we have unambiguously identified for the first time the coexistence of two fluid phases in these ternary mixtures using diffraction techniques. One of these phases is rich in DPPC and the other is rich in DOPC. They are called the liquid ordered (l_o) and liquid disordered (l_d) phases, respectively. The phase behaviour of these ternary mixtures obtained from the diffraction study are in agreement with those reported from earlier fluorescence microscopy studies on GUVs made from these mixtures.

In **chapter 1** we give a brief introduction to lipids and to the various lamellar phases exhibited by them in aqueous solutions. A summary of earlier studies on the influence of cholesterol on lipid membranes and a brief introduction to membrane rafts are also given.

Lipids are the basic structural units of plasma membranes. Phospholipids and sphingolipids are the most common lipids found in these membranes. In general, lipids are amphiphilic molecules, consisting of two parts; a polar hydrophilic head and a nonpolar hydrophobic hydrocarbon chain(s). Lipids in aqueous solution self assemble to form a variety

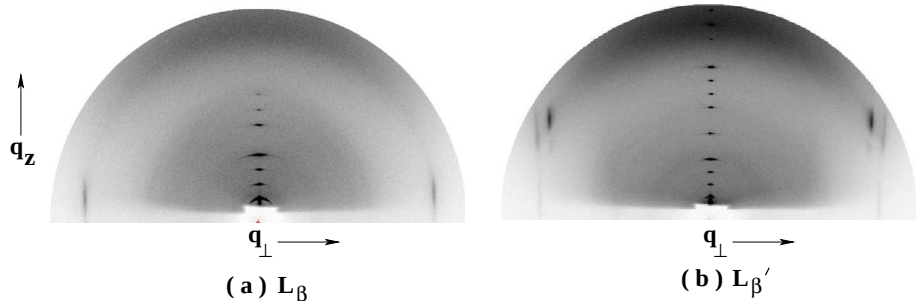


Figure 1: Diffraction patterns of gel phases of DLPE (a) and DPPC (b).

of liquid crystalline phases above the critical micellar concentration (CMC). Typical CMCs of bilayer forming lipids are in the range of $\sim 10^{-6} - 10^{-10}$M.

At high hydration lipids, such as DPPC, exhibit a variety of lamellar phases, depending on the temperature. They show a fluid (L_α) phase above the chain melting transition temperature (T_m), also known as main transition. In this phase hydrocarbon chains are molten and disordered. At lower temperatures, L_α transforms into a gel phase (Fig. 1). In this phase hydrocarbon chains are predominantly in the fully stretched all *trans* conformational state, and form a quasi hexagonal lattice in the plane of the bilayer, as evidenced from the sharp wide angle reflections (Fig. 1). Lipids, such as DLPE, exhibit the L_β gel phase, where the chains are along the bilayer normal (Fig. 1 a). Lipids which have a larger head group, such as DPPC, show the $L_{\beta'}$ gel phase, where the hydrocarbon chains are tilted with respect to the bilayer normal as indicated by the wide angle chain reflections at $q_z \neq 0$ (Fig. 1 b). Some PCs exhibit a ripple ($P_{\beta'}$) phase in between L_α and $L_{\beta'}$ phases. The $P_{\beta'}$ phase is characterized by a two dimensional oblique lattice formed by height modulated bilayers (Fig. 2 a).

Cholesterol is also an essential constituent of plasma membranes. Incorporation of cholesterol into lipid membranes leads to the progressive decrease in main- and pre-transition temperatures and enthalpies with increasing X_c. Below T_m, the gel phase is found to coexist with a cholesterol–rich phase for $\sim 5 < X_c <\sim 20$. The latter phase is known as liquid ordered (l_o) phase in the literature. Although spectroscopic studies, such as nuclear magnetic resonance (NMR), have found a fluid–fluid coexistence above T_m in a similar range of X_c, there has been no evidence for such a coexistence from diffraction studies. These two fluid

phases are the cholesterol–rich l_o phase and the cholesterol–poor liquid disordered (l_d) phase. At sufficiently large X_c (> 20 mol%), the main- and pre-transitions disappear, resulting in a single fluid (L_α) phase rich in cholesterol. In the l_o phase the chain conformational order (membrane fluidity), lateral diffusion and bilayer bending rigidity were found to have an intermediate value between the high temperature fluid (L_α) phase without cholesterol and the low temperature gel phase.

Model membranes composed of ternary mixtures of a saturated lipid, an unsaturated lipid and cholesterol have been widely used to mimic biological membranes. Cholesterol–rich lipid domains are believed to exist in the plasma membranes. However, there is no direct evidence for such domains in these membranes. On the other hand, GUVs made from above ternary mixtures exhibit fluid–fluid immiscibility. One of the fluid phases enriched in the saturated lipid is thought to have a composition similar to that of membrane rafts. This phase is also known as liquid ordered (l_o) phase. The other is rich in unsaturated lipids and is known as liquid disordered (l_d) phase. The biological membrane is a very complex system due to the presence of a variety of lipids and proteins. Various active processes occurring on the cell surface make the situation even more complicated. Further, it is not clear from the present literature whether the rafts that are proposed in plasma membranes are equilibrium structures or maintained by some active processes. Therefore, we do not know how good the analogy between domains in raft mixtures and membranes rafts is. Nonetheless, it is important to establish the behaviour of the simpler system in order to have a better understanding of the much more complex biological membranes.

In **chapter 2** we describe the experimental techniques employed by us for studying lipid–cholesterol membranes. We also discuss basic principles of x-ray diffraction and confocal fluorescence microscopy.

We have mainly used oriented samples for x-ray diffraction study. Samples were prepared on a curved glass substrate where bilayers are aligned parallel to the substrate. All experiments with aligned samples were done at fixed relative humidity (RH) at various tem-

peratures. A locally built humidity controlled chamber is used to collect the diffraction data. The incident monochromatic x-ray beam (wavelength = 1.54 Å) from a rotating anode generator was tangential to the substrate and diffraction patterns were recorded on a two dimensional image plate detector.

The calculation of the electron density map from the observed diffraction data requires both the magnitude of structure factors and their phases. Magnitude of the structure factors were obtained from the experiments. We have used a modeling approach to determine the phases of the reflections. Since the bilayer has a center of symmetry, phases are restricted to be 0 or π. We have observed a modulated (P_β) phase (Fig. 2 b) in PC–cholesterol mixtures, whose structure is somewhat similar to that of the $P_{\beta'}$ phase (Fig. 2 a). This phase will be described in detail in chapter 3. Intensity corrections relevant to this phase are required in order to put all the reflections on the same intensity scale. They are discussed in this chapter. Structure factors calculated from the model were fitted to observed ones to determine the model parameters, such as bilayer thickness and amplitude of the modulation. The calculated phases were combined with the observed magnitudes, and inverse Fourier transformed to get the electron density map.

In this chapter we also describe electroformation of giant unilamellar vesicles (GUVs) made from lipid–cholesterol mixtures. Electroformation is a protocol to prepare GUVs of 10-100 μm size, which can be easily observed under a phase contrast or fluorescence microscope. Vesicles form when the lipid is in the fluid (L_α) phase. We have designed a temperature controlled chamber for the electrofromation so that GUVs can be prepared using lipids which have T_m above room temperature.

In **chapter 3** we present results of our x-ray diffraction studies on oriented multilayers of binary mixtures of cholesterol with DPPC and DMPC. Data were collected at 98 and 75% RH, at temperatures varying from 50°C to 5°C in steps of 5°C. Phases were determined from their characteristics diffraction patterns. The coexistence of two phases was detected from non-overlapping reflections in the diffraction pattern coming from the individual phases.

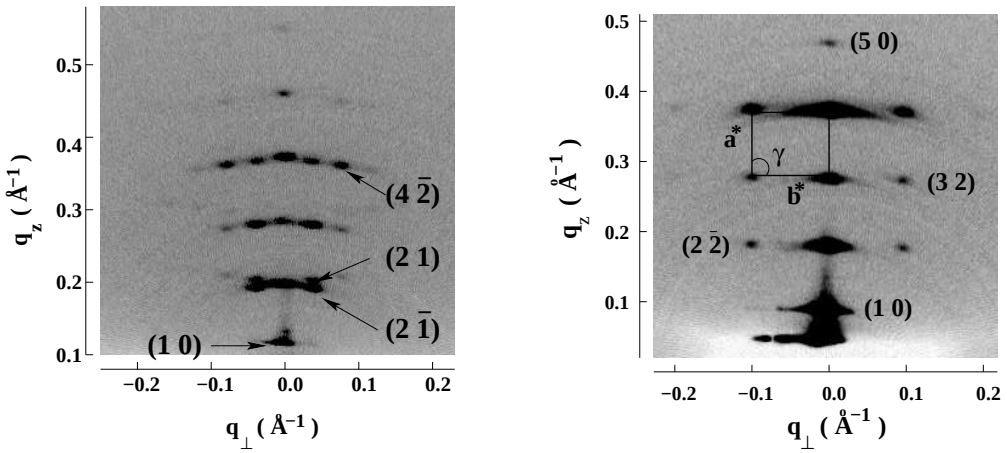
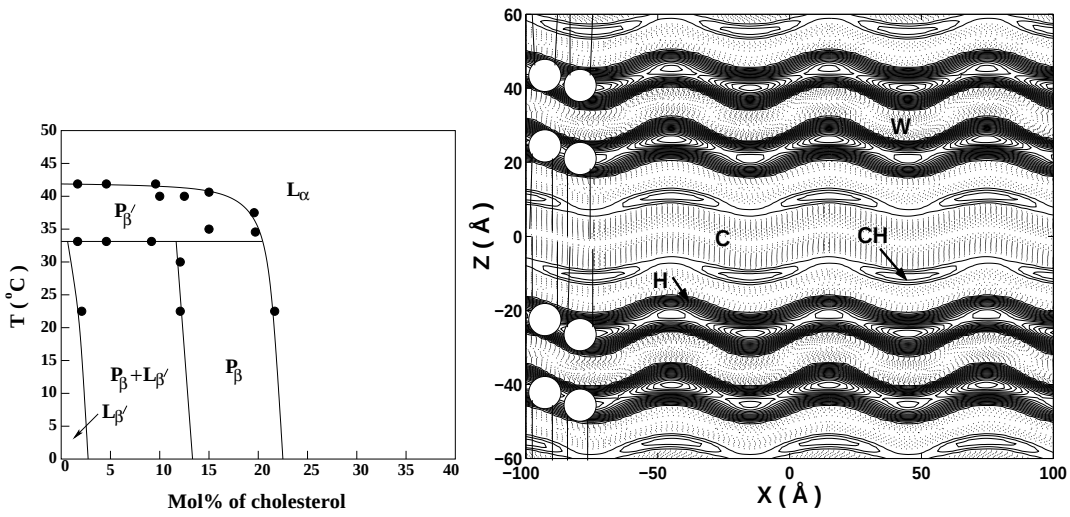


Figure 2: (a) Diffraction pattern of the ripple ($P_{\beta'}$) phase in DMPC bilayers. Reflections can be indexed on an oblique lattice as shown. (b) Diffraction pattern of the P_β phase in a DPPC–cholesterol mixture (X_c = 15 mol%). Reflections can be indexed on a primitive rectangular lattice as shown.

At high hydration DPPC exhibits three lamellar phases, as discussed above. Incorporation of cholesterol into DPPC bilayers leads to an increase in the wavelength of the ripples in the $P_{\beta'}$ phase. This result is consistent with earlier freeze fracture and neutron scattering studies. We have observed the coexistence of gel and the modulated (P_β) phases below $P_{\beta'}$ at intermediate values of X_c. P_β phase was identified from the satellite reflections as shown in Fig. 2 b. At higher X_c, main- and pre-transitions disappear and only the cholesterol–rich l_o phase was found to exist throughout the temperature range studied. The P_β - l_o transition appears to be continuous, as we have not encountered any coexistence region between them. These results are summarized in the partial phase diagram shown in Fig. 3 a. At 75% RH, the $P_{\beta'}$ phase is absent, in agreement with earlier studies. Surprisingly the P_β phase is stabilized at this low RH and occurs over a wide range of temperature from 45°C to 5°C. The electron density map of the P_β phase, calculated from the observed diffraction data is shown in Fig. 3 b. It shows that these bilayers have a rather small height modulation, with an amplitude of ~ 2.5 Å, which is about 5 times smaller than that seen typically in the $P_{\beta'}$ phase. An electron rich band seen at a distance of about 10 Å from the bilayer center is due to cholesterol. It is evident from the map that the two arms of the ripple are of the same length and the bilayer thickness in them is comparable.



(a) (b)

Figure 3: (a) Phase diagram of DPPC–Cholesterol mixtures at 98% RH, determined from the diffraction data. (b) Electron density map of the P_β phase of DPPC–cholesterol mixtures. The solid (dotted) contours correspond to the electron rich (poor) regions of the bilayer. H, W and C denote the head group, water and chain regions of the bilayer, respectively. CH denotes the electron rich band in the bilayer due to the presence of cholesterol. X_c = 15 mol%, T = 6°C, RH = 98 %, d = 66.3 Å, λ = 60.7 Å. The arrangement of the lipid molecules in the bilayer is schematically shown in (b).

The P_β phase (Fig. 2 b) differs in many ways from the ripple ($P_{\beta'}$) phase (Fig. 2 a). First of all, $P_{\beta'}$ is well known to occur only at very high RH, close to 100 %. In contrast, P_β occurs even at 75 % RH. Secondly, the variation of the modulation wavelength (λ) with cholesterol content shows opposite trends in the two phases. In the $P_{\beta'}$ phase λ increases with X_c and seems to diverge near $X_c \sim 20$ mol%. On the other hand, in the P_β phase it decreases with X_c and tends to zero at a similar concentration. In contrast to the oblique lattice in the $P_{\beta'}$ phase, P_β phase has a rectangular lattice. In the P_β phase, λ ($\sim$ 70 Å) is comparable to the bilayer periodicity ($\sim$ 66 Å), whereas it is $\sim$ 150 Å in the $P_{\beta'}$ phase. Unlike the $P_{\beta'}$, the P_β phase has no average chain tilt.

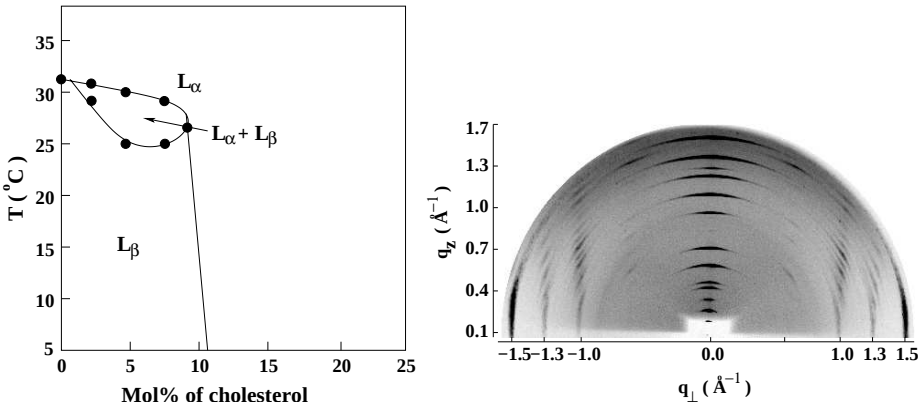
The P_β phase observed here has not been reported in any of the earlier studies. Very small amplitude of the bilayer height modulations in this phase is one likely reason for its not being seen in freeze fracture studies. Unoriented samples of these mixtures show a large d-spacing ($\sim$ 80 Å) in the intermediate cholesterol concentration range, indicating flexible bilayers in this phase. It is possible that on swelling the height modulation of the bilayers are

no more correlated. Therefore, the P_β phase might not exist in excess water.

The formation of the P_β phase at intermediate X_c is rather intriguing. We suspect that the miscibility gap between the $L_{\beta'}$ and P_β phases is a consequence of the non-zero chain tilt in the former. As is well known, the chain tilt arises from the larger cross-sectional area of the head group compared to that of the chains. Therefore, if the tilt can take only values close to 30° and 0° (as observed experimentally), it is conceivable that a well-defined amount of cholesterol has to be incorporated into the bilayer to remove the tilt. A rough estimate based on the areas of the different moieties gives $X_c \approx 20$ mol% in the untilted phase. Therefore, for $X_c < 20$ mol%, the cholesterol distribution in the bilayer may be nonuniform with cholesterol free regions alternating with regions where $X_c \approx 20$ mol%. Such a nonuniform distribution of cholesterol is seen in the electron density map (Fig. 3 b), where the distribution of cholesterol alternates between the two monolayers, making the bilayer locally asymmetric. Such an asymmetry can lead to a non-zero local spontaneous curvature of the bilayers. Therefore, the height modulation in the P_β phase might be the consequence of local spontaneous curvature due to the out of phase periodic localization of cholesterol in the two monolayers making up a bilayer.

The fluid–fluid immiscibility above T_m detected using spectroscopic studies such as NMR has not been observed in any diffraction study. Although we have observed a single lamellar phase, there could still be a nonuniform distribution of cholesterol, such as microscopic domains of different chain conformational order. These microscopic domains can easily be detected using NMR. However, small angle diffraction studies cannot detect such domains due to negligible contrast between the two phases. Condensed wide angle reflections above T_m of the lipid in the l_o phase indicate the stretching of the chains in the presence of cholesterol. The presence of large number of lamellar reflections in this phase can be understood in terms of the higher rigidity of the bilayer at high X_c.

In **chapter 4** we describe the phase behaviour of DLPE–cholesterol mixtures. Pure DLPE exhibits the fluid (L_α) to gel(L_β) phase transition at $\sim$ 30°C. For 2.5< X_c < 10, the L_α was



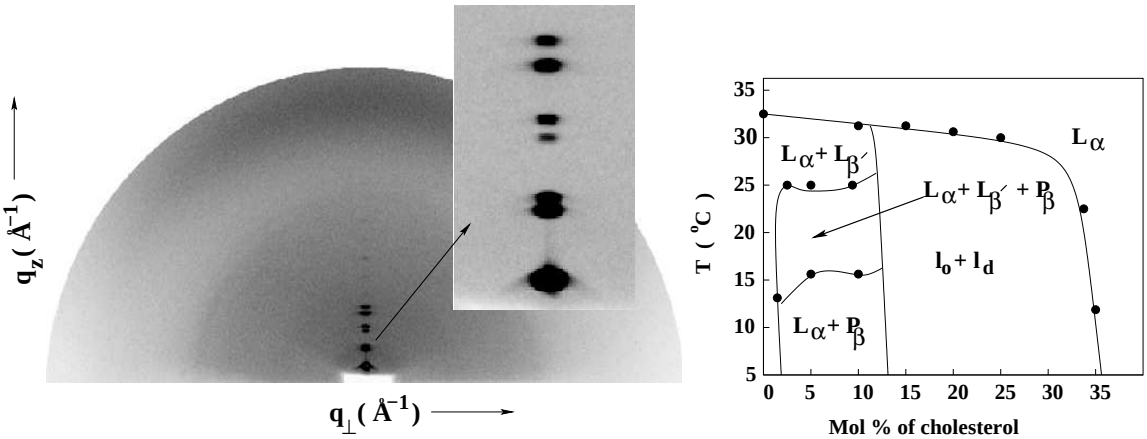
(a) (b)

Figure 4: (a) Phase diagram of DLPE-Cholesterol mixtures at 98% RH, determined from the diffraction data. (b) Diffraction pattern of a highly ordered phase of DLPE–cholesterol mixtures (X_c = 10 mol%) observed before heating to high temperatures.

found to coexist with the L_β below T_m over a narrow range of temperature. At lower temperatures L_β was observed throughout the temperature range studied. At $X_c > 10$ mol%, a cholesterol–rich L_α phase was found at all temperatures. A partial phase diagram obtained from our diffraction data at 98% RH is shown in Fig. 4 a. One interesting observation in DLPE–cholesterol mixtures is the formation of a highly ordered phase as shown in Fig. 4 b. This phase is usually formed after long incubation at low temperature ($\sim 4^\circ$C). The presence of cholesterol seems to facilitate the formation of this phase.

DLPE–cholesterol mixtures do not exhibit the P_β phase, indicating the role of chain tilt in the formation of this phase. It is known that the $P_{\beta'}$ phase occurs only in lipids which have non zero chain tilt. Our results indicate that the formation of the P_β phase too is confined to similar lipids. Formation of the P_β phase could be the consequence of the fact that gel phase in PC–cholesterol mixtures can take very small amount of cholesterol (< 2.5 mol%), whereas the gel phase can incorporate about 10 mol% of cholesterol in DLPE–cholesterol mixtures.

In **chapter 5** we describe the influence of cholesterol on DOPC bilayers. The motivation for studying DOPC–cholesterol mixtures was to understand the behaviour of fluid l_d phase which is known to be one of the coexisting phases in ternary raft mixtures. DOPC is

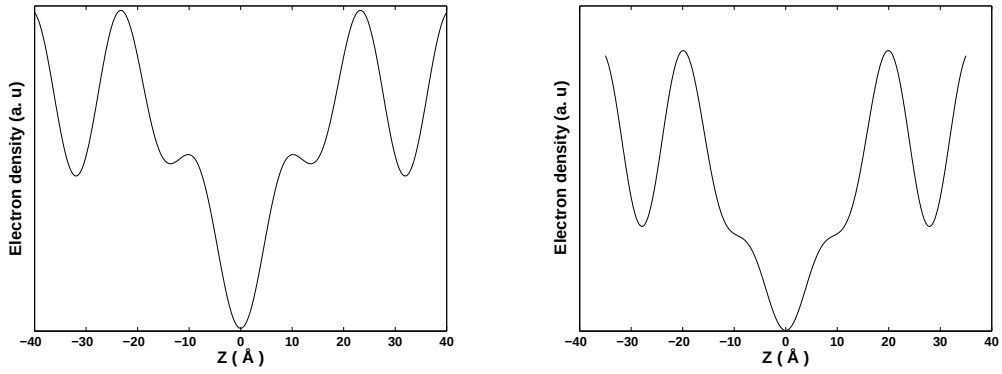


(a) (d = 56.4 ; 61.3 Å) (b)

Figure 5: (a) Small angle region of the diffraction pattern showing the coexistence of l_o and l_d phases in an equimolar mixture of DPPC, DOPC and cholesterol at 10°C. The d-spacings corresponding to the two lamellar structures are also indicated. (b) Pseudo binary phase diagram of equimolar mixtures of DPPC and DOPC at different cholesterol concentrations derived from x-ray diffraction data.

an unsaturated lipid and the main transition temperature is about -18°C. DOPC–cholesterol mixtures show a single fluid (L_α) phase throughout the temperature range studied. Since in the present experimental setup it is not possible to reach temperatures below ∼5°C, we cannot access the gel phase of DOPC. We have constructed the transbilayer electron density profiles from the observed diffraction data. There is no significant change in these electron density profiles as a function of X_c. However, the broad trough seen in these profiles due to the lower electron density of the terminal methyl group gets narrowed as X_c is increased due to the presence of cholesterol.

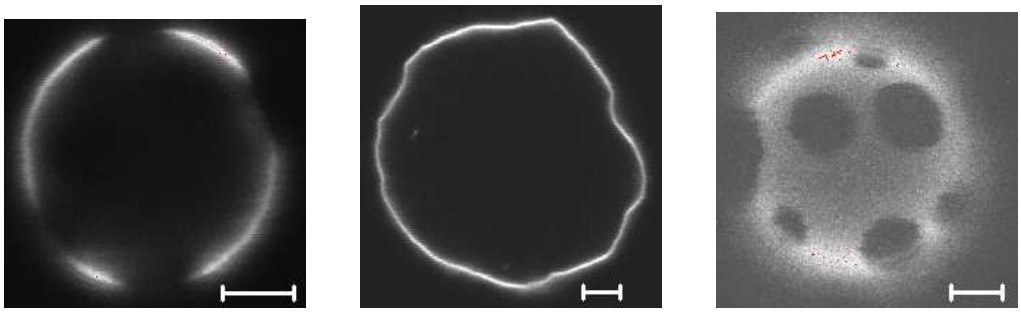
In **chapter 6** we present our experimental results on ternary mixtures composed of DPPC, DOPC and cholesterol. These mixtures are known to be relevant to the formation of rafts. An important result of these studies is the observation of the coexistence of two fluid phases for $15 \leq X_c \leq 35$ in these mixtures (Fig. 5 a). Although the fluid–fluid immiscibility was seen in giant unilamellar vesicles made up of ternary raft mixtures using fluorescence microscopy, we have observed for the first time such a coexistence using diffraction. In both the coexisting phases wide angle reflections are too weak to be detected, indicating fluid phases



(a) (b)

Figure 6: (a) Electron density profiles of the l_o (a) and l_d (b) phases obtained from an equimolar mixture of DPPC and DOPC by adding cholesterol (X_c = 25 mol%, T = 10°C).

(Fig. 5 a). However, for small values of X_c, the fluid (L_α) was found to coexist with the gel ($L_{\beta'}$) due to the large difference in T_m of DPPC and DOPC. We also observed three phase coexistence of L_α, $L_{\beta'}$ and the P_β phase. A pseudo binary phase diagram obtained from the diffraction data is presented in Fig. 5 b. Comparing the electron density profiles of the two fluid phases (Fig. 6) with those obtained from binary mixtures of cholesterol with DPPC and DOPC, it is clear that one of the coexisting phases is rich in DPPC and other rich in DOPC. The phase with larger d-spacing is identified as DPPC–rich l_o phase and the other as the DOPC–rich l_d phase. However, cholesterol contents of these two fluid phases cannot be determined from our present diffraction data. It is interesting to note that the d-spacing of the l_o phase decreases, whereas it increases in the l_d phase as X_c is increased, indicating that both phases contain significant amount of cholesterol. At X_c = 35 mol%, both the d-spacings are comparable. These results suggest that there exists a threshold value of X_c, above which the coexistence disappears. The threshold value of X_c decreases with increasing temperature. It is possible that there is a critical point at which the fluid–fluid immiscibility transition is continuous. Presently we cannot determine the critical point as we have only studied one slice of the ternary phase diagram. At $X_c > 35$ mol%, we have observed a single fluid phase, consistent with earlier microscopy observations. Similar behaviour was also observed in equimolar mixture of sphingomyelin, DOPC and cholesterol.



(a) (b) (c)

Figure 7: (a, b) Equatorial sections of GUVs made from a DPPC–cholesterol mixture (X_c = 5 mol%) obtained from confocal fluorescence microscopy. The smaller GUV in (a) shows gel - L_α coexistence, whereas the larger one in (b) does not, but exhibits significant thermal shape fluctuations. (c) Top section of a GUV, made from a ternary equimolar mixture of DPPC, DOPC and cholesterol, showing l_o - l_d coexistence. Scale bars, 5 μm.

In **chapter 7** we present our confocal fluorescence microscopy observations on giant unilamellar vesicles (GUVs) made up of binary and ternary lipid–cholesterol mixtures. All experiments were done at room temperature (23°C). GUVs were prepared using electroformation, as described in chapter 2, and were labeled with the fluorescent dye Rhodamine DHPE and LAURDAN. LAURDAN is an environment sensitive probe whose excitation and emission spectra depend on the solvent polarity, and lipid phase state. In the gel phase LAURDAN emits at ~ 440 nm (blue emission), but emission spectrum gets red shifted to ~ 490 nm in the fluid phase. Relative intensities of blue and red shifted emission is a measure of lipid phase state and are used to obtain the generalized polarization, defined as $GP = \frac{I_{440}-I_{490}}{I_{440}+I_{490}}$. In practice GP is low (~ 0.2) in the fluid phase and high (~ 0.8) in the gel phase. A laser scanning confocal fluorescence microscope was used to obtain images of GUVs (Fig. 7).

Small GUVs (diameter < ~ 20 μm) are almost spherical in shape and exhibit phase separation for $1.5 \leq X_c \leq 10$. Dark irregular domains seen in the bright background are rigid and do not coalesce, indicating that they are in the gel phase (Fig. 7 a). However, at these X_c, larger GUVs do not show domains, but exhibit significant thermal shape fluctuations, indicating the softening of the bilayers in the presence of cholesterol (Fig. 7 b). This observation is supported by the fact that GUVs show a low value of mean GP, suggesting increase in the fluidity of the membranes. The swelling behaviour of unoriented samples at these X_c, lead-

ing to a large d-spacing (~ 80 Å) substantiates these microscopy observations. Asymmetric distribution of cholesterol in the two monolayers (as evidenced from the electron density map of the P_β phase) can in principle change the local spontaneous curvature, leading to a shape deformation. The coupling between the local concentration fluctuations and curvature can also lower the bending rigidity.

At $X_c > 10$ mol%, GUVs are mostly spherical in shape and do not show significant thermal shape fluctuation, indicating increase in bending rigidity. Laurdan GP also shows a high value (~ 0.8) which is close to that seen in the gel phase. However, GUVs made up of DMPC–cholesterol mixtures show non spherical shapes, but do not exhibit significant thermal shape fluctuations.

GUVs made from ternary raft mixtures show back circular domains on a bright background (Fig. 7 c). These circular domains are mobile and eventually coalesce and form large domains of diameter ~ 10 μm, indicating that they are in the fluid phase. These two fluid phases often referred to as l_o and l_d phases. The line tension at the boundary between the two coexisting phases drives the formation of bigger domains. Both the phases are capable of forming buds with no curvature preference. This is consistent with earlier two-photon fluorescence microcopy on GUVs. Our microscopy results are in broad agreement with those obtained from our diffraction studies on the same mixtures.

The following papers contain the work described in this thesis:

1. Cholesterol-induced modulated phase in phospholipid membranes,
   Sanat Karmakar and V. A. Raghunathan,
   Phys. Rev. Lett. **91**, 98102 (2003).

2. Phase behaviour of dipalmitoyl phosphatidylcholine (DPPC)–cholesterol membranes,
   Sanat Karmakar, V. A. Raghunathan, and Satyajit Mayor,
   J. Phys.:condens. Matter **17**, S1177 (2005).

3. Structure of phospholipid–cholesterol membranes: an x-ray diffraction study,
   Sanat Karmakar and V. A. Raghunathan
   (to appear in *Phys. Rev. E*, 2005).

4. Observation of fluid–fluid immiscibility in ternary mixtures of dipalmitoyl phosphatidylcholine (DPPC), dioleoyl phosphatidylcholine (DOPC) and cholesterol,
   Sanat Karmakar, V. A. Raghunathan, and Satyajit Mayor
   (to be submitted).