Chapter 4

Mixed monolayer of cholesterol and thiocholesterol

4.1 Introduction

Cholesterol (Ch) is an important biological molecule [1, 2]. Thiocholesterol (TCh) differs from the Ch molecule only on the replacement of -OH group by -SH group at its 3β position. There are reports on the formation of defect rich self-assembled monolayer (SAM) of TCh on gold substrates. The size of the such defects are of the order of 5-8 Å [3]. Such defect rich SAM can be utilized as ultramicroelectrodes and selective permeation devices. It can be potentially used in electroanalytical and biosensor applications. However, the limitation of the formation of SAM of such organosulfur compounds is the substrate which has to be of coinage metals like gold, silver and copper. Another method of obtaining monolayer and multilayer on different types of substrate is Langmuir-Blodgett (LB) technique. Here, the monolayer is transferred to any solid substrate from the air-water (A-W) interface. Hence, the formation of a stable Langmuir monolayer is important for the formation of controlled and organized LB films. It is well known that the Ch molecules form a stable monolayer at the A-W interface [4, 5]. Langmuir monolayer and the LB films of Ch and its mixture with fatty acids and phospholipids have been extensively studied [5,6]. Though -SH group is weakly acidic in nature [7], the amphiphilic balance of the TCh for the formation of a stable insoluble monolayer at the A-W interface is not sufficient. The TCh molecule is mostly hydrophobic in nature and does not spread to form a monolayer at the A-W interface.

In the previous chapter, we have shown that the cholesterol molecules mix readily in the cholesteric acid monolayer. There, the miscibility of the molecules was attributed to the van der Waals and hydrophobic interactions between the sterol moiety and the ease of hydrogen bond formation of the polar groups of the molecules. In this chapter, we describe our study on the role of hydrophobic sterol moiety on stabilizing the mixed monolayer of cholesterol and its thiol analogous, thiocholesterol.

4.2   Experimental

The molecule, cholest-5-ene-3β-thiol (thiocholesterol) was obtained commercially from Aldrich. Thiocholesterol (TCh) was used as procured. The solutions of the samples having a concentration of 2.59 mM were prepared in HPLC grade chloroform and the mixture was prepared by mixing them in appropriate quantities. The monolayer at the air-water interface was compressed at a rate of 4.3×10^{-2} ($\mathring{A}^2$/molecule)/s. The experimental details for the surface manometry and the microscopy were similar as discussed in the previous chapters.

4.3   Results

The surface pressure (π) - area per molecule (A_m) isotherms for the different mole fractions of TCh in the mixed monolayer of Ch and TCh (X_{TCh}) are shown in Figure 4.1. The isotherm of cholesterol shows the coexistence of a gas and an untilted condensed (L_2) phase at a large A_m. At an A_m of 39 $\mathring{A}^2$, there is a steep rise in the surface pressure indicating a transition from the coexistence of gas and L_2 phases to the L_2 phase. The limiting area per molecule (A_o) is equal to 38 $\mathring{A}^2$. This value approximately corresponds to the cross-sectional area of the Ch molecule for its normal orientation at the A-W interface. The monolayer collapses at an A_m of 37 $\mathring{A}^2$ with a collapse pressure of 43 mN/m. The Ch monolayer shows a spike-like collapse and a plateau thereafter [5]. Being a hydrophobic material, the TCh molecules do not spread at the A-W interface and do not show any surface pressure on compression. We have attempted to form the TCh monolayer on different subphases obtained by changing the pH and adding salts in the ultrapure ion-free water. However, we were not able to form a
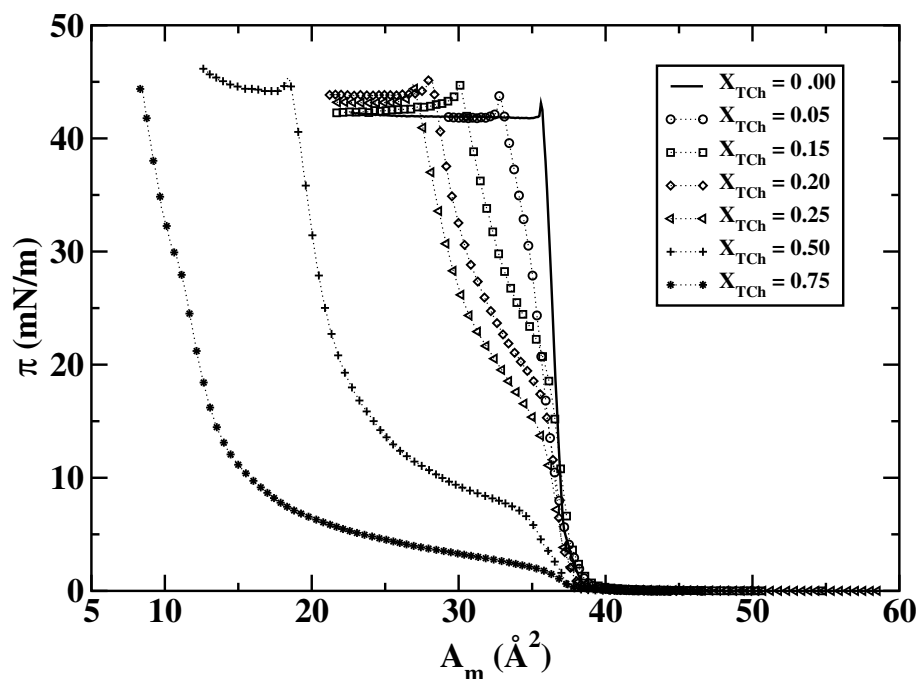
Figure 4.1: The surface pressure (π) - area per molecule (A_m) isotherms of the mixed monolayer for different mole fractions of TCh in Ch (X_{TCh}).

stable TCh monolayer over such aqueous subphases. The isotherms of the mixed monolayer (Figure 4.1) show a sharp rise in surface pressure at around 39 $\mathring{A}^2$. However, the presence of TCh in Ch monolayer changes the nature of the isotherm by introducing an additional change in slope in the isotherms. Such change in slope can be considered as an initial collapse of the two-component monolayer system. The isotherms also show a final collapse. The final collapse of the mixed monolayer reveals spike-like feature followed by a plateau. This is characteristic of the collapse for the cholesterol monolayer. Hence, the final collapse indicates a collapse of the Ch rich monolayer. The A_o values for the mixed monolayer were found to be nearly invariant with X_{TCh}. It lies in the range of 37.5 - 38.5 $\mathring{A}^2$. Figure 4.2 shows a variation of the collapse pressures (π_c) as a function of the mole fraction of TCh in Ch (X_{TCh}). Since the pure TCh does not show any rise in surface pressure on compression, it has been assumed that the value of collapse pressure for pure TCh is zero. The initial collapse pressure decreases continuously with the increase in X_{TCh}. It lies in the range of the collapse pressures of pure individual components. This indicates a miscible nature of the mixed components [8, 9]. The values of the final collapse pressure remain independent
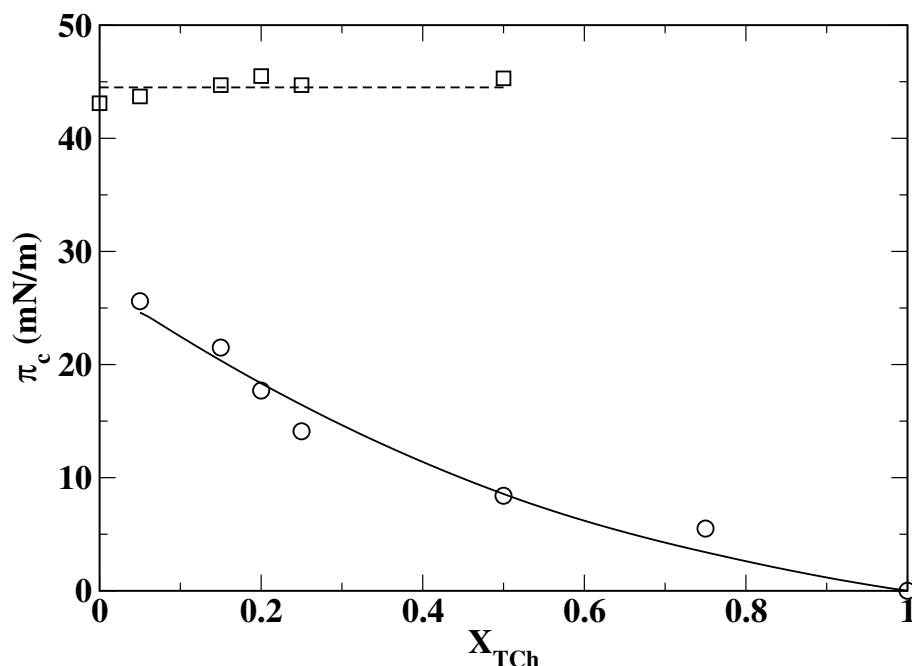
Figure 4.2: Variation of collapse pressure (π_c) with mole fraction of TCh in Ch (X_{TCh}). The solid line with circles represents the initial collapse of the mixed monolayer. The dotted line with squares represents the final collapse.

with X_{TCh}. The nature of the final collapse and its variation with X_{TCh} indicate that the final collapse is due to the collapse of the Ch rich monolayer.

The Brewster angle microscope (BAM) images of the mixed monolayer for the two different mole fractions of TCh in Ch are shown in Figure 4.3. At a large A_m, the mixed monolayer shows the coexistence of dark and gray regions (Figures 4.3(a) and 4.3(d)). The dark region represents the gas phase, whereas the gray domains represents a condensed phase. On compression, the dark region disappears and a homogeneous gray texture of the condensed phase was observed (Figures 4.3(b) and 4.3(e)). Further compression of the monolayer leads to the appearance of small bright domains in the gray background (Figures 4.3(c) and 4.3(f)). This is the initial collapse of the mixed monolayer.

The epifluorescence microscope experiments were performed on the mixed monolayer of Ch and TCh for various X_{TCh}. The epifluorescence results are consistence with those obtained from surface manometry and BAM results. At a large A_m, a coexistence of dark region and a gray region was observed (Figures 4.4(a) and 4.4(d)). The gray region was liquid like. The dark region represents the gas phase, whereas the gray region represents
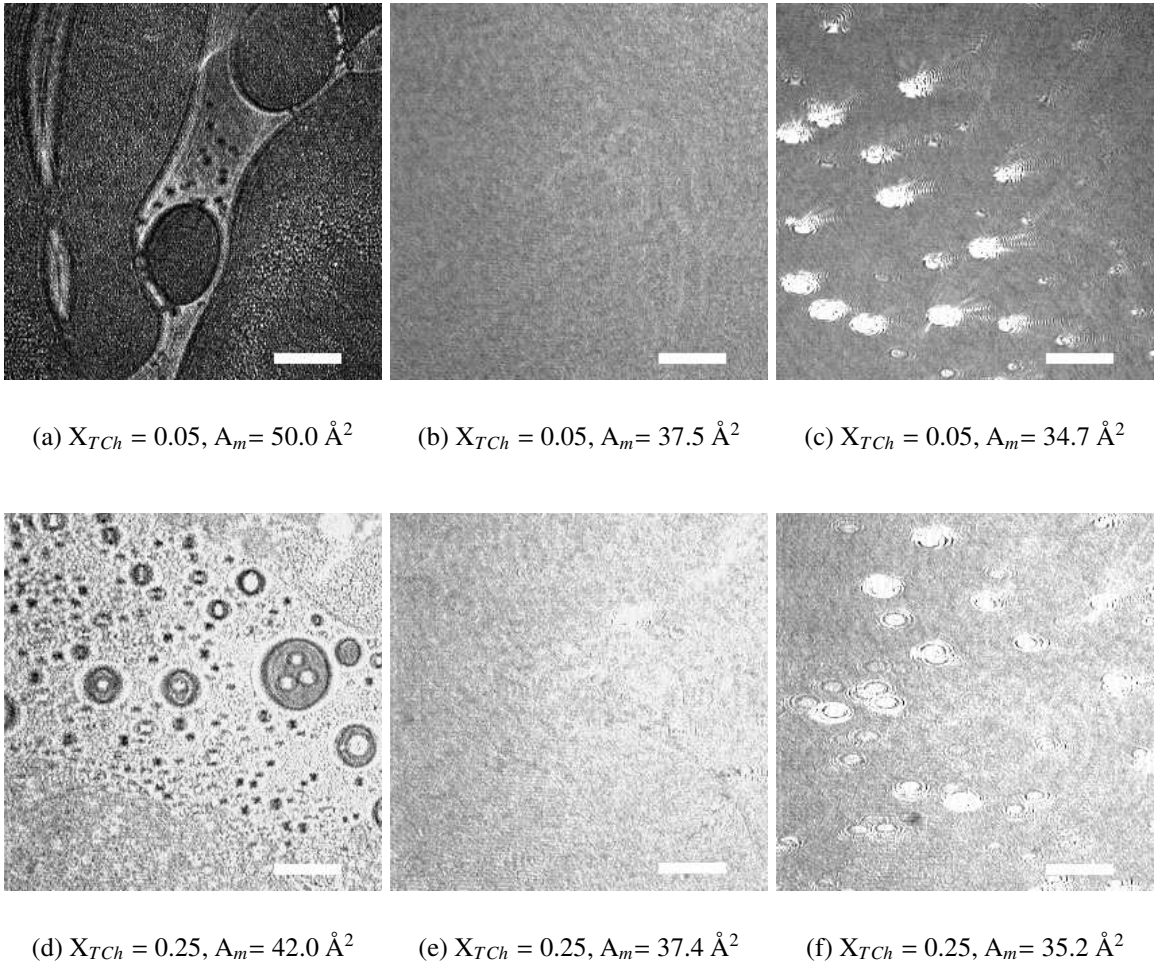
(a) $X_{TCh} = 0.05$, $A_m = 50.0$ Å^2 (b) $X_{TCh} = 0.05$, $A_m = 37.5$ Å^2 (c) $X_{TCh} = 0.05$, $A_m = 34.7$ Å^2

(d) $X_{TCh} = 0.25$, $A_m = 42.0$ Å^2 (e) $X_{TCh} = 0.25$, $A_m = 37.4$ Å^2 (f) $X_{TCh} = 0.25$, $A_m = 35.2$ Å^2

Figure 4.3: BAM images of the mixed monolayer for different mole fractions of TCh in Ch (X_{TCh}). The corresponding A_m and the X_{TCh} values are shown below the respective images. (a) and (d) show the coexistence of dark region (gas phase) and gray domains (a condensed phase). (b) and (e) show a very uniform gray texture of the condensed phase. (c) and (f) represent the images after the initial collapse. Here, small bright domains are coexisting with the uniform gray background. The scale bar represents 500 μm.

the condensed phase of the mixed monolayer. On compression, the dark region vanishes, yielding an uniform gray texture (Figures 4.4(b) and 4.4(e)). On further compression, the monolayer exhibits the initial collapse. Here, the images (Figures 4.4(c) and 4.4(f)) show some bright domains coexisting with the uniform gray background.

The expansion of the monolayer immediately after the initial collapse does not lead to the disappearance of the small bright domains in the BAM and the epifluorescence images. Figure 4.5 shows a BAM image on expansion of the mixed monolayer captured immediately
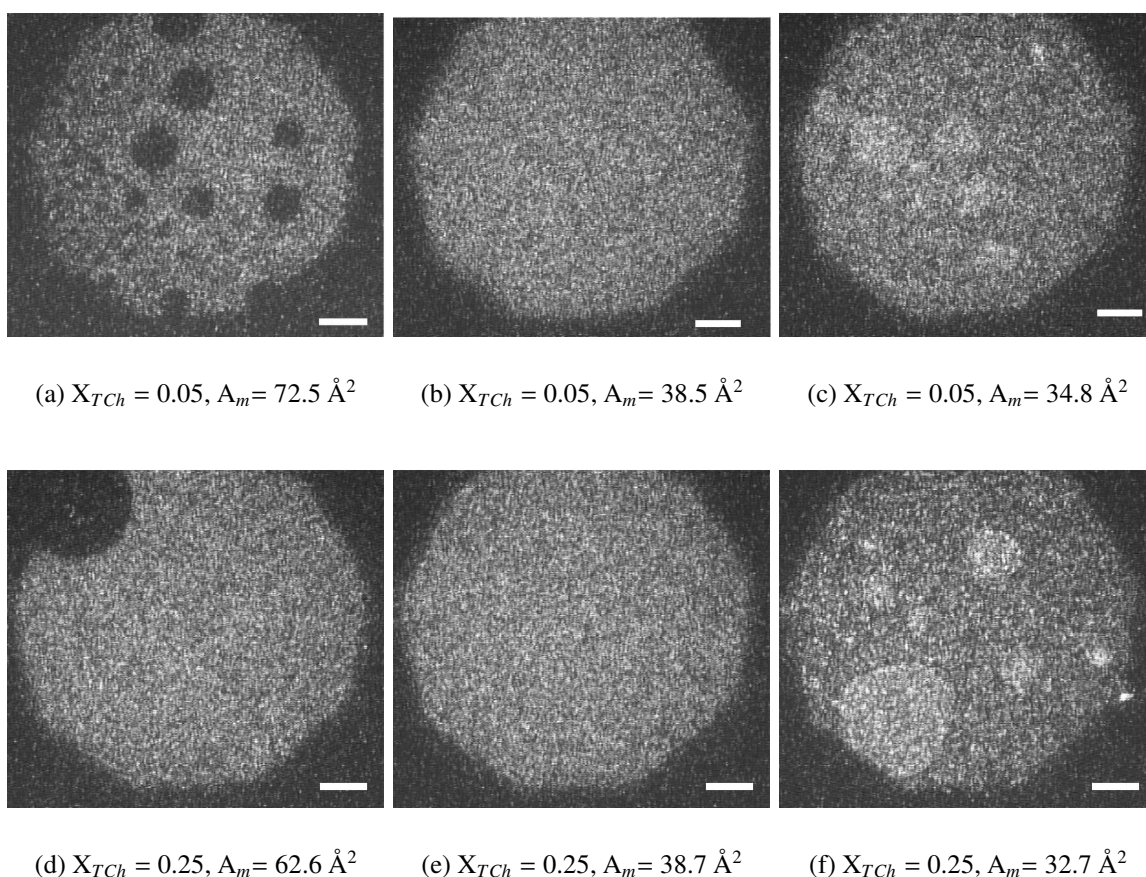
(a) $X_{TCh} = 0.05$, $A_m= 72.5$ Å^2 (b) $X_{TCh} = 0.05$, $A_m= 38.5$ Å^2 (c) $X_{TCh} = 0.05$, $A_m= 34.8$ Å^2

(d) $X_{TCh} = 0.25$, $A_m= 62.6$ Å^2 (e) $X_{TCh} = 0.25$, $A_m= 38.7$ Å^2 (f) $X_{TCh} = 0.25$, $A_m= 32.7$ Å^2

Figure 4.4: Epifluorescence images of the mixed monolayer for different mole fractions of TCh in Ch (X_{TCh}). The corresponding A_m and the X_{TCh} values are shown below the respective images. (a) and (d) show a coexistence of dark region (gas phase) and a gray region (a condensed phase). (b) and (e) show a very uniform gray texture of the condensed phase. (c) and (f) represent the images after the initial collapse. Here, small bright domains are coexisting with the uniform gray background. The scale bar represents 50 μm.

after initial collapse. Here, a coexistence of dark region (gas phase), gray region and bright domains are seen.

4.4   Discussion

Cholesterol forms a stable Langmuir monolayer at the A-W interface. However, the sterol possessing hydrophobic TCh molecule does not spread at the A-W interface. The mixed monolayer of Ch and TCh shows a steep rise in surface pressure on compression. The values of limiting area per molecule (A_o) suggest a normal orientation of the molecules in the phase corresponding to steep region of the isotherms (Figure 4.1). The phase in this
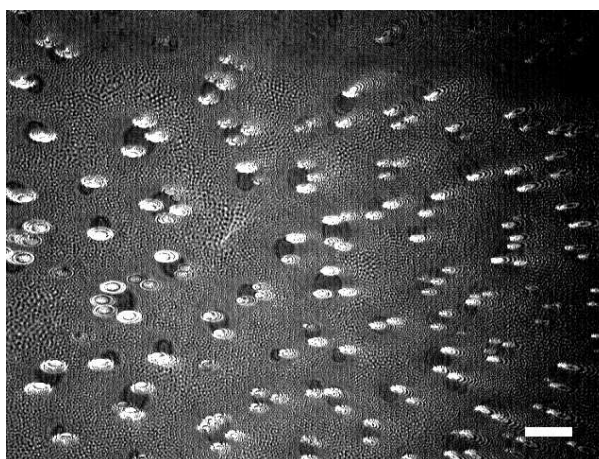
Figure 4.5: The BAM image during the expansion of the mixed monolayer having $X_{TCh} = 0.05$ after its initial collapse. The image was taken at an A_m of 70 $Å^2$. The scale bar represents 500 μm.

region of the isotherms may represent untilted condensed (L_2) phase. The BAM images (Figures 4.3(b) and 4.3(e)) reveal a very uniform texture in this region of the isotherms suggesting an uniform orientation of the component molecules. The epifluorescence images (Figures 4.4(b) and 4.4(e)) also show an uniform gray texture in this region of the isotherm. This indicates a miscible nature of the component molecules. After the initial collapse, the BAM and epifluorescence images reveal bright domains coexisting with an uniform gray background. The brightness indicates that the domains are sufficiently thick. The bright domains do not vanish on expansion of the monolayer after the initial collapse (Figure 4.5). This indicates the spreading incapability of the bright domains at the A-W interface. We expect that due to compression, the non-amphiphilic TCh molecules to squeeze out from the mixed monolayer at the initial collapse. The bright domains represent the domains of squeezed out TCh molecules. After the initial collapse, the uniform background in the images (Figures 4.3(c) and 4.4(f)) represent a homogeneous phase. On further compression, this phase also collapses. The final collapse behavior is similar to that of Ch monolayer. Hence, the homogeneous phase after the initial collapse may represent a Ch rich phase. The rise in surface pressure after the initial collapse of the mixed monolayer is due the Ch rich monolayer.

Figure 4.6 shows the variation of the peak values of in-plane elastic modulus (E_p)
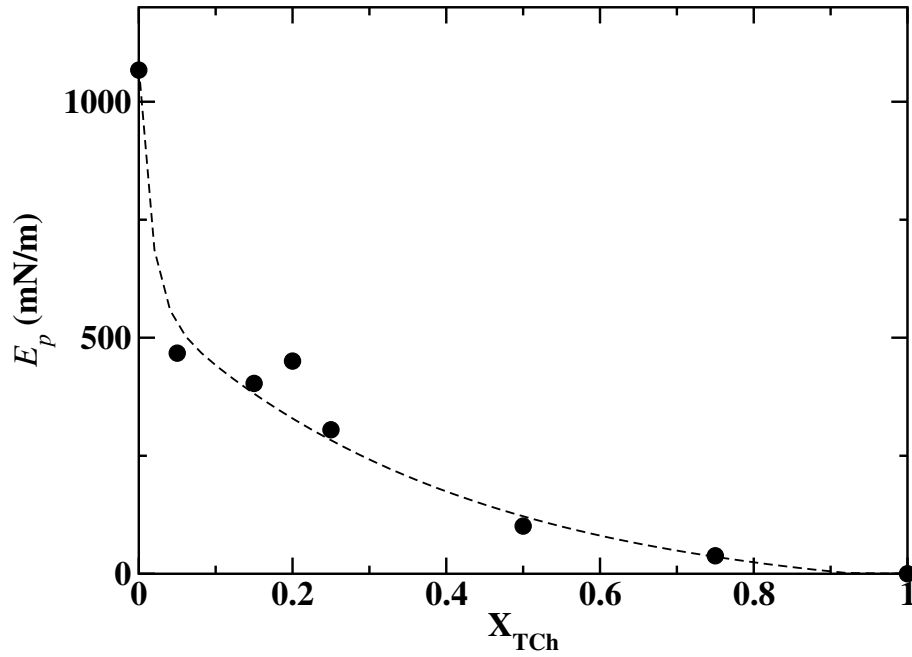
Figure 4.6: Variation of the peak value of in-plane elastic modulus (E_p) in the untilted condensed (L_2) phase with mole fraction of the TCh in Ch (X_{TCh}).

in the untilted condensed (L_2) phase with X_{TCh}. The E_p for the Ch monolayer was 1076.0 mN/m. The E_p value of the mixed monolayer for X_{TCh} equal to 0.05 decreases sharply to 467.8 mN/m. It goes to a minimum value of 38 mN/m for the X_{TCh} equal to 0.75. The L_2 phase of the Ch monolayer is highly rigid in nature. However, the presence of even 0.05 mole fraction of TCh in Ch reduces the E_p value of the condensed phase by a factor of half. E_p varies slowly thereafter and finally shows a very small value. Hence, the condensed phase of the mixed monolayer is more fluidic as compared to that of pure Ch monolayer.

For an ideal case of the two-component system of non-interacting molecules, the area per molecule of the mixed monolayer at a given surface pressure (π) is given by the rule of additivity [10]

$$A_{id} = X_1 A_1 + X_2 A_2 \tag{4.1}$$

where A_{id} is the A_m of an ideal mixed monolayer, A_1 and A_2 are the area per molecule of the components 1 and 2, and X_1 and X_2 are the mole fractions of the components 1 and 2, respectively. If one of the components is a non-amphiphilic molecule (for instance

82

component 2), then the Equation 4.1 can be modified as

$$A_{id} = X_1 A_1 = (1 - X_2)A_1 \qquad (4.2)$$

The Equation 4.2 indicates that A_{id} varies linearly with the mole fraction of either of the components. The excess area per molecule (A_{ex}) at a given surface pressure (π) can be defined as

$$A_{ex} = A_{12} - A_{id} \qquad (4.3)$$

where A_{12} is the experimentally determined area per Ch molecule for the mixed monolayer at a constant surface pressure (π). The negative or the positive value of A_{ex} suggests an attractive or a repulsive interaction between the component molecules, respectively. The
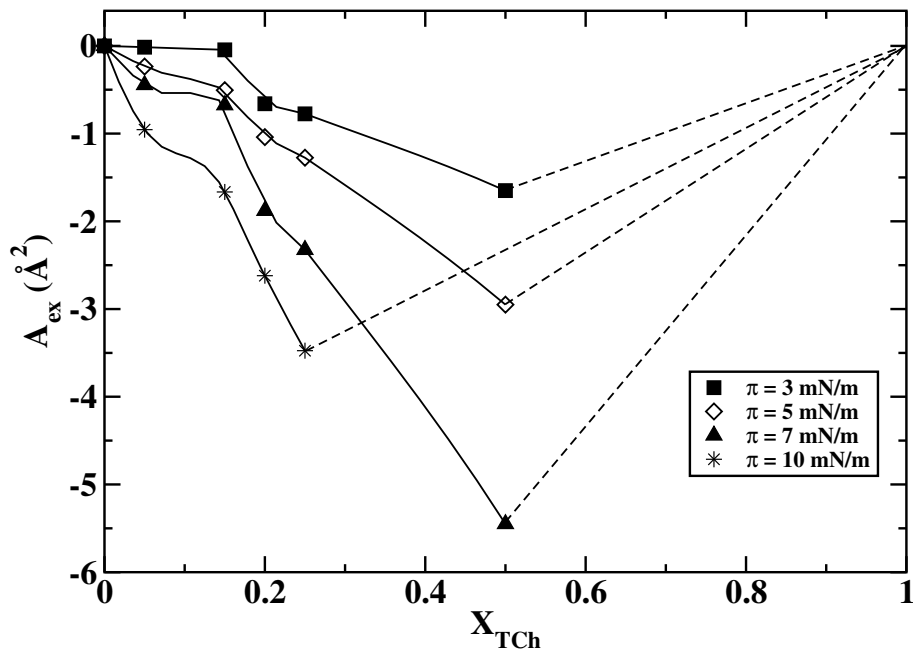

Figure 4.7: Variation of excess area per molecule (A_{ex}) with respect to the mole fraction of TCh in Ch (X_{TCh}) at different surface pressures (π). The points are computed using Equation 4.3. The dashed lines are extrapolated to the zero value of the excess area for the pure TCh.

variation of excess area per molecule (A_{ex}) as a function of mole fraction of TCh in Ch (X_{TCh}) for different surface pressures is shown in Figure 4.7. The values of A_{ex} are negative for all the compositions. The negative values of A_{ex} suggest an attractive interaction between the Ch and TCh molecules in the mixed monolayer. Such attractive interaction leads to an stabilization effect on the mixed monolayer of Ch and TCh.

There are many reports on pure hydrophobic materials forming a stable monolayer at the A-W interface [11,12]. Tabe *et al.* [12] have reported the formation of condensed monolayer of pure hydrophobic liquid crystals at the A-W interface. They attributed the stability of the monolayer to the gain in entropy on adsorption of the molecules at the interface. Li *et al.* [11], have suggested that the stability of purely hydrophobic materials like a long chain alkane can be due to the van der Waals force. There is another report which demonstrated the stability of alkanes monolayer at the A-W interface. This has been attributed to the gain in entropy due to strong fluctuations of the alkanes on a plane normal to interface as compared to their states in the bulk solid [13]. In the present case, we assume that the stability of the mixed monolayer of Ch and TCh is due to the van der Waals and hydrophobic interactions between the sterol moiety. There are possibilities for the gain in entropy due to the rearrangement of the interfacial water molecules and the fluctuation of the molecules as compared to their state in the bulk solids. Jensen *et al.* [14, 15], have shown experimentally and through simulation that the orientation of interfacial water molecules change differently due to the presence of hydrophobic or hydrophilic molecule at the interface. The dipole moment associated with the water molecule faces on an average towards the hydrophobic alkane. However, for the hydrophilic monolayer covered interface, it faces away from the interface. We assume that for a mixed system where the head groups of the two mixed components are different, the individual molecules orient the interfacial water molecules differently. This will lead to a frustration in orientation of the water molecules at the interface which may reduce the ordering of the interfacial water dipole moments. This may lead to an increase in the entropy of the system which in turn may help in stabilizing the mixed monolayer. Hence, we can assume the entropy gain in the system is due to the reorientation of the water molecules at the interface and the fluctuations of the iso-octyl chain of the Ch and TCh molecules along the plane normal to the interface. On reducing the intermolecular distances by compressing the monolayer, the steric repulsion among the molecules is sufficient enough to overcome the van der Waals attraction and the entropy gain. These may lead the non-amphiphilic component (*i.e.* TCh) to squeeze out of the mixed monolayer. We present a simple model describing the
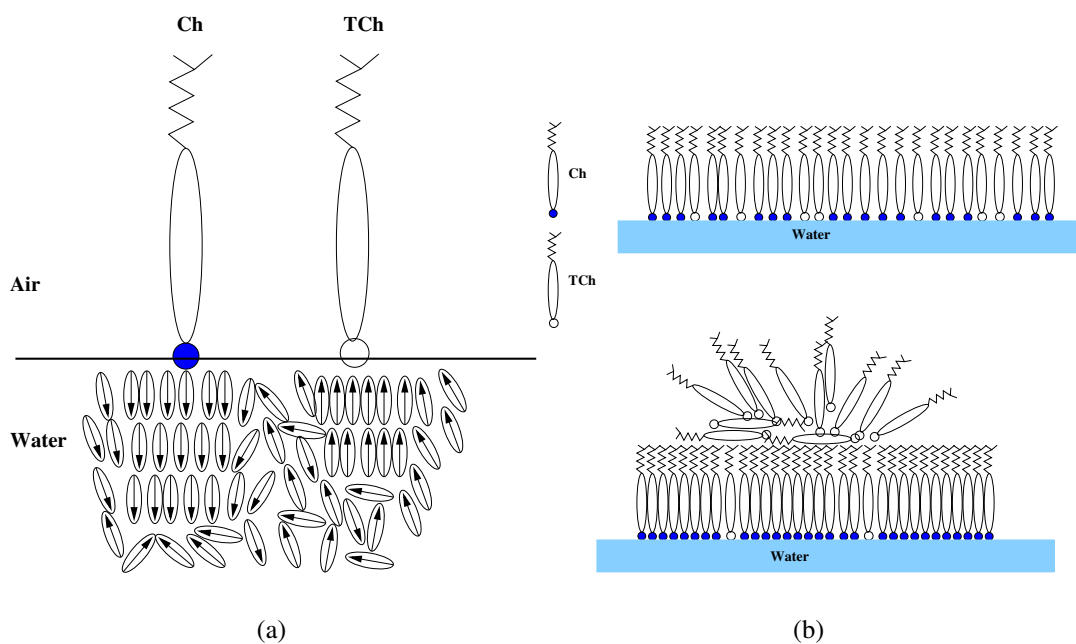
Figure 4.8: (a) a simple model describing the orientation of the water at the interface in the presence of Ch and TCh. The arrow in the water molecules represent the direction of dipole moment. (b) shows orientation of the Ch and TCh molecules in the L_2 phase and the squeezing out of the TCh molecules after the initial collapse.

squeezing out of the TCh molecule from the mixed monolayer due to compression. This is shown in Figure 4.8.

Figure 4.8 depicts a simple model showing the orientation of the water molecules due to presence of -OH group of Ch and -SH group of TCh molecule at the A-W interface. The orientations of the water dipole moment are shown in opposite direction for Ch and TCh molecules. Due to such opposite orientation, the water molecule may get disordered at some regions of the interface. This may lead to increase in entropy of the system. In addition, the fluctuation of the hydrophobic iso-octyl chain of Ch and TCh molecules may also contribute to the increase in entropy. However, when the intermolecular distances decrease to some critical value, the non-amphiphilic component (*i.e.* TCh) gets squeezed out. The squeezed out domains stay on the cholesterol rich monolayer, as shown in Figure 4.8(b). These thick nucleated domains of TCh were unable to spread on expansion.

4.5 Conclusions

There are numerous reports in literature stating the formation of mixed monolayer of a non-amphiphilic component into the monolayer of an amphiphilic molecule [16–21]. In all these cases, the monolayer has been stabilized upto certain value of surface pressure due to van der Waals and hydrophobic interactions of the tail groups of the different components. Further compression leads to the squeezing out of the non-amphiphilic components. Our studies reveal that TCh molecules do not spread on aqueous subphase and form a stable insoluble monolayer. However, we were successful in stabilizing the TCh molecules in the cholesterol monolayer. We find that the mixed monolayer for X_{TCh} upto 0.75 give rise to surface pressure. The mixed monolayer is stable upto the initial collapse pressure. Above the initial collapse, the TCh molecules squeeze out from the mixed monolayer. There is a final collapse which is due to the breakdown of the cholesterol rich monolayer. We find the phases shown by mixed monolayer were gas, L_2 and collapsed state. Our studies suggest an attractive interaction between the molecules which can be attributed to the van der Waals and hydrophobic interactions between the sterol moiety of the molecules.

Bibliography

[1] Philip L. Yeagle, Biochim. Biophys. Acta **822**, 267 (1985).

[2] R. Lipowsky and E. Sackmann, *Structure and dynamics of the membranes* (North-Holland, Amsterdam, 1995).

[3] Z. P. Yang, I. Engquist, J. -M. Kauffmann, and B. Liedberg, Langmuir **12**, 1704 (1996).

[4] J. P. Slotte and P. Mattjus, Biochim. Biophys. Acta **1254**, 22 (1995).

[5] S. Lafont, H. Rapaport, G.J. Sömjen, A. Renault, P.B. Howes, K. Kjaer, J. Als-Nielsen, L. Leiserowitz, and M. Lahav, J. Phys. Chem. B **102**, 761 (1998).

[6] K. S. Birdi, *Lipid and Biopolymer Monolayers at Liquid Interfaces* (Plenum, New York, 1989).

[7] R. Bilewicz and M. Majda, Langmuir **7**, 2794 (1991).

[8] G. L. Gaines, Jr., *Insoluble Monolayers at Liquid-Gas Interfaces* (Wiley-Interscience, New York, 1966).

[9] G. Roberts, *Langmuir-Blodgett Films* (Plenum, New York, 1990).

[10] A. W. Adamson, *Physical Chemistry of Surfaces* (Wiley-Interscience, New York, 1990).

[11] M. Y. Li, A. A. Acero, Z. Huang, and S. A. Rice, Nature (London) **367**, 151 (1994).

[12] Y. Tabe, T. Yamamoto, I. Nishiyama, K. M. Aoki, M. Yoneya, and H. Yokoyama, J. Phys. Chem. B **106**, 12089 (2002).

[13] A. V. Tkachenko and Y. Rabin, Phys. Rev. Lett. **76**, 2527 (1996).

[14] T. R. Jensen, M. Ø. Jensen, N. Reitzel, K. Balashev, G. H. Peters, K. Kjaer, and T. Bjørnholm, Phys. Rev. Lett. **90**, 086101 (2003).

[15] M. Ø. Jensen, O. G. Mauritsen, and G. H. Peters, J. Chem. Phys. **90**, 9729 (2004).

[16] A. M. Gonçalves da Silva, J. C. Guerreiro, N. G. Rodrigues, and T. O. Rodrigues, Langmuir **12**, 4442 (1996).

[17] A. K. Dutta, T. N. Mishra, and A. J. Pal, Langmuir **12**, 459 (1996).

[18] A. K. Dutta, Langmuir **12**, 5909 (1996).

[19] H. Wang, Y. Ozaki, and K. Iriyama Langmuir **16**, 5142 (2000).

[20] P. Viswanath and K. A. Suresh, Langmuir **20**, 8149 (2004).

[21] F. Nannelli, V. S. U. Fazio, Y. Matsuzawa, L. Komitov, and K. Ichimura, http://xxx.lanl.gov/pdf/cond-mat/0010482.