

# Comparison of physical models used to explain condensation effects in lecithin-cholesterol mixed films

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**ABSTRACT** The apparent condensation effect observed with mixed monolayers of lecithin and cholesterol has been examined with respect to both molecular interaction and molecular cavity models. For each, model equations are presented which predict the surface areas of the film components as a function of the mole fraction of each component. For mixed monolayers where linearity does not exist in plots of mean surface area vs. mole fraction, and where there are breaks in the curve at mole fraction values other than 0.5, the size of the cavities are not invariant with respect to mole fraction.

**SUPPLEMENTARY KEY WORDS** mixed monolayers · surface pressure · molecular cavities · intermolecular interaction

**P**HOSPHOLIPIDS and cholesterol are major components of biological membranes which are composed of bi-molecular leaflets or lipoprotein subunits (1). Force-area curves of mixed monolayers have been used widely as physical models for studying lipid-lipid interactions that may have relevance to those interactions occurring at the membrane surface. Mixed films of lecithin and cholesterol were first studied by de Bernard (2) who noted that cholesterol apparently was able to condense lecithin to a smaller cross-sectional area than it can exhibit alone. Van Deenen, Houtsmuller, DeHaas, and Mulder (3) verified this observation and indicated the biological significance of these findings.

On the other hand, Shah and Schulman (4) explained the apparent condensation of mixed monolayers of lecithin and cholesterol by a cavity effect. These molecular cavities or vacancies are caused by thermal motion of

the fatty acyl chains of the lecithin molecules. The size of the cavities is determined by the surface pressure of the film for any given fatty acyl chain. The condensation effect is then due to the fact that the cholesterol molecule can occupy the cavity and cause no proportional increase in the area per molecule of the mixed monolayer.

A condensation effect, whether due to molecular interaction or to a cavity effect, is seen when the following relation is obeyed at constant surface pressure:

$$A_m < A_1^0 (1 - X_2) + A_2^0 X_2 \quad (1)$$

where  $A_m$  is the mean area per molecule of the mixed film,  $A_1^0$  and  $A_2^0$  are the areas per molecule of pure lecithin and cholesterol, respectively at this surface pressure, and  $X_2$  is the mole fraction of cholesterol. Values of  $A_1^0$  and  $A_2^0$  are obtained from surface pressure-surface area ( $\pi$ - $A$ ) plots of pure lecithin and pure cholesterol films, respectively, whereas values of  $A_m$  are obtained from  $\pi$ - $A$  plots of the various mole fractions of mixed lecithin-cholesterol films.

The purpose of this report is to examine both the interaction model and the molecular cavity model, particularly as a function of mole fraction of lecithin and cholesterol.

The interaction model, whether due to a stoichiometric complex or to a molecular realignment, assumes that at constant surface pressure the molecular area of cholesterol is constant at all mole fractions and that the condensation effect is always due to a decrease in the molecular area of the lecithin molecules (see reference 3). Thus at constant surface pressure:

$$A_1 = (A_m - A_2^0 X_2) / (1 - X_2) \quad (2)$$

On the other hand, the molecular cavity model assumes that at constant surface pressure, the molecular area of lecithin and, hence, the size of the cavities are constant at all mole fractions. Any observed condensation effect is then always a function of the cholesterol within these cavities resulting in a decrease in the average area of the surface occupied by the cholesterol molecules (4). Thus, at constant surface pressure:

$$A_2 = (A_m - A_1^0 [1 - X_2])/X_2 \quad (3)$$

Finally, the percentage reduction of the area per lecithin molecule ( $P_L$ ) based on the interaction model, and the percentage decrease in the surface area occupied by the cholesterol molecule ( $P_c$ ) based on the molecular cavity model can be calculated as:

$$P_L = ([A_1^0 - A_1]/A_1^0) 100 \quad (4)$$

and

$$P_c = ([A_2^0 - A_2]/A_2^0) 100 \quad (5)$$

respectively. It should be kept in mind that increases in values of  $P_L$  and  $P_c$  correspond to decreases in the surface area occupied by lecithin and cholesterol, respectively.

The values of  $A_1$ ,  $A_2$ ,  $P_L$ , and  $P_c$  were calculated for the dipalmitoyl lecithin-cholesterol system using the data of Shah and Schulman (4). Plots of  $P_L$  vs.  $\pi$  and  $P_c$  vs.  $\pi$  for the various mole fractions are shown in Figs. 1A and B, respectively.

If we first consider the interaction model (Fig. 1A) we see that the value of  $P_L$  decreases as a function of surface pressure, finally reaching a value of 0 at 30 dynes/cm. The value of  $P_L$  also increases as a function of  $X_2$ , reaching a limiting value at any given surface pressure, at a value of  $X_2 = 0.5$ . Thus, an increase in the mole fraction

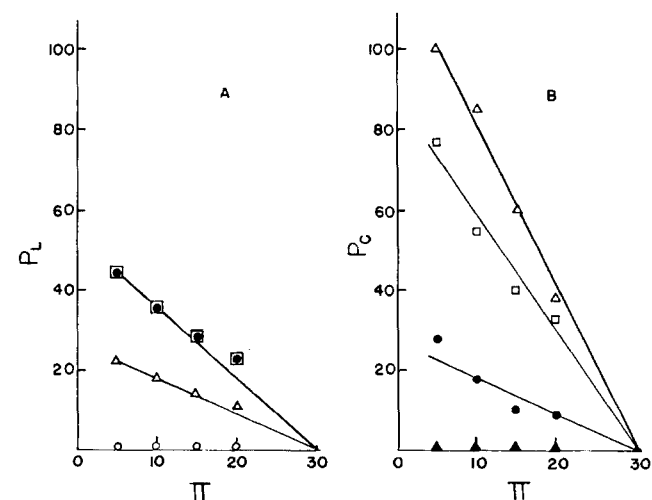


FIG. 1. (A)  $P_L$  vs.  $\pi$  (interaction model) and (B)  $P_c$  vs.  $\pi$  (molecular cavity model) for various mole fractions of cholesterol ( $X_2$ ):  $X_2 = 0$ ,  $\circ$ ;  $X_2 = 0.25$ ,  $\triangle$ ;  $X_2 = 0.50$ ,  $\square$ ;  $X_2 = 0.75$ ,  $\bullet$ ;  $X_2 = 1.0$ ,  $\blacktriangle$ .

of cholesterol from 0.5 to higher values has no effect on the degree of condensation. This is apparent from the  $\pi$ - $A$  curves for dipalmitoyl lecithin-cholesterol mixed films at  $X_2$  values of 0.5, 0.67, 0.75, and 1.0 (Fig. 4 of reference 4). We can thus schematically represent the structure of the monolayers at any surface pressure value by using the areas of cholesterol and lecithin as calculated from the values of  $A_2^0$  and  $P_L$  ( $A_1^0$ ), respectively. Such a schematic representation is shown for various mole fractions of cholesterol at a surface pressure of 10 dynes/cm in Fig. 2A-D. It can be seen that the area occupied per lecithin molecule will decrease as a function of the mole fraction of cholesterol until a value of  $X_2 = 0.5$  is reached. At this mole fraction, the condensation effect is maximal, and further additional cholesterol will not affect the area per molecule occupied by lecithin.

Differentiation of eq. (2) with respect to  $X_2$  will yield:

$$dA_m/dX_2 = dA_1/dX_2 - (dA_1/dX_2)X_2 - A_1 + A_2^0 \quad (6)$$

At mole fractions where the  $\pi$ - $A$  curves are superimposable ( $X_2 \geq 0.5$ ),  $dA_m/dX_2$  and  $dA_1/dX_2 = 0$ . Thus  $A_1 = A_2^0$  as is depicted in Figs. 2C-D.

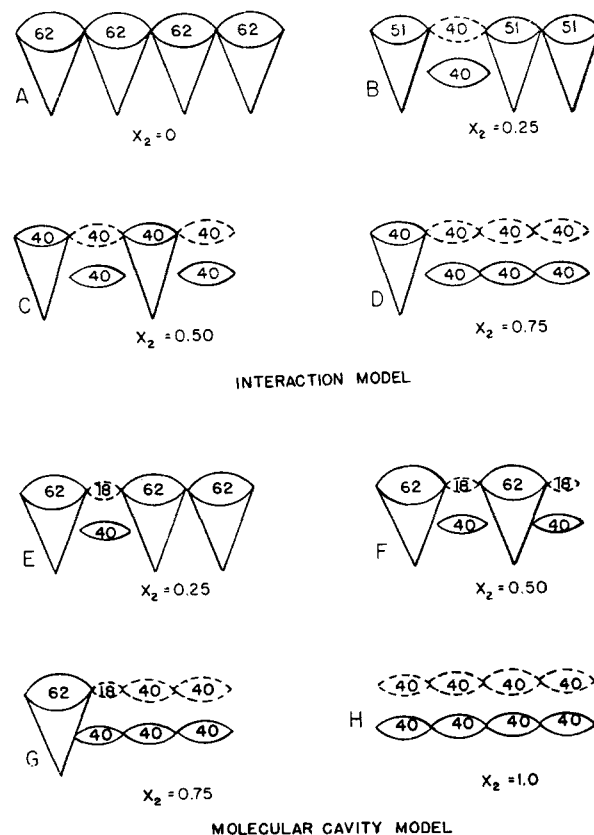


FIG. 2. Schematic representation of structure of monolayers using the interaction model and the molecular cavity model for various mole fractions of cholesterol ( $X_2$ ). Lecithin,  $\nabla$ ; cholesterol,  $\circ$ ; area per molecule occupied at the surface by cholesterol,  $\therefore$ .

If we next consider the molecular cavity model (Fig. 1B) we see that the value of  $P_c$  also decreases as a function of surface pressure, again reaching a value of 0 at 30 dynes/cm. The value of  $P_c$  increases as a function of  $X_1$  at any given surface pressure but does not appear to reach a limiting value, as in the case of the interaction model.

We can again schematically represent the structure of the monolayers at any surface pressure value by using the areas of cholesterol and lecithin as calculated from the values of  $P_c(A_1^0)$  and  $A_2^0$ , respectively. Such a schematic representation is shown for various mole fractions of cholesterol at a surface pressure of 10 dynes/cm in Figs. 2E–H. At  $X_2 \leq 0.5$ , the average surface area occupied per cholesterol molecule is  $18 \text{ \AA}^2$ , the remainder of the molecule being in the cavity. At higher values of  $X_2$ , all excess cholesterol molecules (those in excess of the number of lecithin molecules present) must occupy their full molecular area ( $40 \text{ \AA}^2$ ), since there are no cavities available for them. The model predicts that the area per lecithin molecule, as well as the space available for cholesterol in the cavity, is invariant with respect to  $X_2$  at constant surface pressure. Therefore, values of  $P_c$  should be constant at all values of  $X_2$  up to 0.5 if one assumes that one cholesterol molecule fits into each cavity.

Differentiation of eq. (3) by  $X_2$  will yield:

$$dA_m/dX_2 = A_1^0 + (dA_2/dX_2)X_2 + A_2 \quad (7)$$

At mole fractions where there are sufficient cavities for the cholesterol ( $X_2 \leq 0.5$ ) the size of the cavities should be invariant with respect to  $X_2$  and  $dA_2/dX_2 = 0$ .

Thus,  $dA_m/dX_2 = A_2 - A_1^0$  and  $A_2$  will be constant only when a plot of  $A_m$  vs.  $X_2$  is linear at values of  $X_2$  from 0 to 0.5. Furthermore, such a plot should yield a break at  $X_2 = 0.5$ , since at this point all of the cavities are occupied. Such is the case with the data presented by Shah and Schulman (4).

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