# Interaction of Cholesterol and Octadecanol in a Mixed Adsorbed Film at Carbon Tetrachloride/Water Interface: Criticism about the Condensing Effect of Cholesterol

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In order to make clear the lateral interaction between sterol nucleus and hydrocarbon chain in a membrane, the mixed adsorbed film of octadecanol and cholesterol at a carbon tetrachloride/water interface was studied by measuring the interfacial tension as a function of the total concentration and composition of the mixture. An adsorbed film has the advantages that the measurements are made under a thermodynamic equilibrium condition and that an expanded state of cholesterol can be examined. The adsorbed film of pure octadecanol formed only an expanded state under the present experimental conditions. On the other hand, the film of pure cholesterol exhibited an expanded/condensed phase transition. The mixed adsorbed film also exhibited the expanded/condensed phase transition when the composition of cholesterol was larger than 0.5. The transition accompanied an abrupt but relatively small change in the interfacial density. It was shown that mixtures are less concentrated than the pure components at the interface and that the partial molar areas of both components have larger values than the molar areas of pure ones. We conclude, therefore, that cholesterol molecules do not act as condensing agents on hydrocarbon chains in the adsorbed film and that the interacting force between the sterol nucleus and hydrocarbon chain is weaker than the one between the nuclei or the chains. Our results indicate that an analysis of the spread monolayer must be made with great caution.

## Introduction

The interaction between sterol nucleus and hydrocarbon chain has been widely studied because of its importance in biology. The interest has been focused on their miscibility and especially on the condensing effect of cholesterol molecules in membranes.[1,2] A variety of lipid chains have been found to show this condensing effect by use of their monolayers spread at an air/water interface.[3-5]

The condensing effect concept is based on monolayer studies. However, the studies have disadvantages in that the cholesterol molecules form a condensed film at the surface and do not mix well with fatty acyl chains in the condensed film.[6] Since biological membranes are usually in a liquid crystalline state, which corresponds to an expanded state of monolayers, the condensed state is not appropriate for consideration of the effect of cholesterol on properties of monolayers. Further, constituents of the monolayer cannot migrate into the bulk phases; the rearrangement of molecules, which is accompanied by a change of the state of membranes, should produce a change in composition in the membrane. Since, in biological membranes, the composition of cholesterol is maintained by a thermodynamic equilibrium,[7] the spread monolayer is not suitable for a study of the mixed system. An additional approach to an examination of the interaction between sterol nucleus and hydrocarbon chain in a membrane is necessary.

We measured here the interfacial tension of carbon tetrachloride solution of octadecanol and cholesterol against water as a function of concentration and composition at 25 °C to clarify the interaction between sterol nucleus and hydrocarbon chain. The carbon tetrachloride/water interface was selected so that the analysis of the expanded film could be made over the whole composition range of cholesterol. The octadecanol molecule was chosen as a representative of the hydrocarbon chain, since most of the previous works have been done by using a surface-active substance with an 18-carbon chain length and the octadecanol molecule has the same hydrophilic group as the cholesterol molecule.

## Experimental Section

Carbon tetrachloride was purified with activated alumina (Wholem Basic Act. I) and then distilled. Water was twice distilled, the second being done from alkaline permanganate solution. Their purity was checked by measuring the interfacial tension between them. The cholesterol had a stated purity of 99+% (Sigma, standard for chromatography) and was used without further purification. Octadecanol, with 99.5% purity (Tokyo Kasei), was recrystallized from the carbon tetrachloride solution. The purity was checked by gas chromatography; only one peak was observed.

The interfacial tension measurements were performed by the pendant drop method as previously described.[8] The temperature of the pendant drop cell was controlled to ±0.01 °C by circulating thermostated water through the jacket of the cell. The temperature of the whole equipment was also held nearly constant at the cell temperature. While 30 min was enough for the adsorption and thermal equilibrium, the pendant drop was allowed to stand for 40 min before the measurement of interfacial tension.

## Results and Discussion

Since the thermodynamic treatment for the competitive adsorption of solutes at the interfaces has been established,[9,10] we measured the interfacial tension as a function

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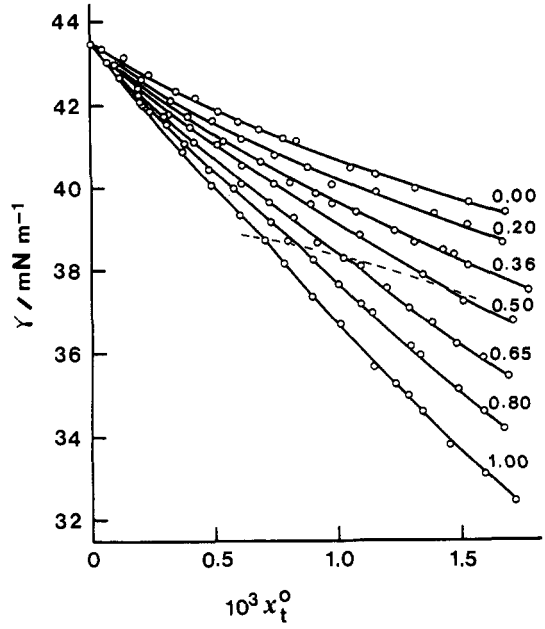


**Figure 1.** Variation of the interfacial tension with total concentration at fixed composition and a temperature of 25 °C. The numbers shown in the figure are the compositions of cholesterol in the oil phase. The dotted line connects the breaks in curves where the phase transition takes place.

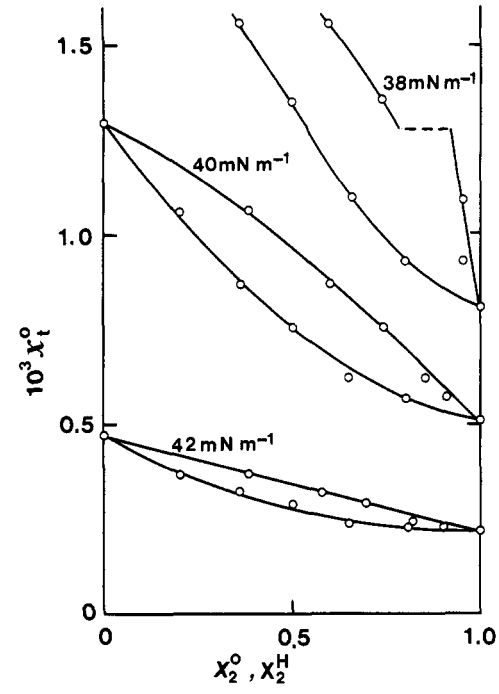


**Figure 2.** Compositions in the oil (lower curves) and mixed adsorbed phase (upper curves) at the fixed interfacial tension values.

of the total mole fraction of octadecanol and cholesterol in carbon tetrachloride, $x_t^o$, and the composition of cholesterol, $X_2^o$, defined as

$$x_t^o = (n_1^o + n_2^o)/(n_0^o + n_1^o + n_2^o) \quad (1)$$

$$X_2^o = n_2^o/(n_1^o + n_2^o) \quad (2)$$

at 25 °C and atmospheric pressure. Here $n$ is the number of moles. Subscripts 0, 1, and 2 refer to carbon tetrachloride, octadecanol, and cholesterol, respectively, and superscript o denotes the carbon tetrachloride phase.

Figure 1 shows the variation of interfacial tension $\gamma$ with $x_t^o$ at fixed $X_2^o$. The top curve, which is that of the pure octadecanol system, exhibits a monotonous decrease in the interfacial tension while increasing the total concentration. In contrast to this curve, the bottom curve for the pure cholesterol system bears a break point which is not as clear as that observed in the case of the adsorption of octadecanol at the hexane/water interface.[11] We have shown that the phase transition takes place in the adsorbed films of surface-active substances at oil/water and air/water interfaces and causes the plots of $\gamma$ vs $x_t^o$, $\gamma$ vs $T$, or $\gamma$ vs $p$ to break at the transition point.[8,11-15] Taking these results into account, the break point in the $\gamma$ vs $x_t^o$ curve of cholesterol is concluded to represent the expanded/condensed phase transition in the adsorbed film. The break of the curve is also observed, when the composition of cholesterol exceeds 0.50, shifting to a dilute total concentration and higher interfacial tension while increasing the composition of cholesterol. The dotted line in Figure 1 expresses the lows of the break point.

According to the thermodynamic treatment described previously,[9,10] the variation of interfacial tension with total concentration and composition at fixed temperature and pressure is expressed as

$$d\gamma = -(RT\Gamma_t^H/x_t^o)dx_t^o - RT(\Gamma_2^H/X_2^o - \Gamma_1^H/X_1^o)dX_2^o \quad (3)$$

In this equation, $\Gamma$ is the interfacial density defined with respect to the two dividing planes which make the interfacial excess numbers of moles of carbon tetrachloride and water zero. The superscript H denotes the interfacial excess quantity. $\Gamma_t^H$ is the total interfacial density given by $\Gamma_t^H = \Gamma_1^H + \Gamma_2^H$. From eq 3, the total interfacial density and composition in the mixed film, $X_2^H$, defined by

$$X_2^H = \Gamma_2^H/\Gamma_t^H \quad (4)$$

are given by

$$\Gamma_t^H = -(x_t^o/RT)(\partial\gamma/\partial x_t^o)_{T,p,X_2^o} \quad (5)$$

$$X_2^H = X_2^o - (X_1^o X_2^o/x_t^o)(\partial x_t^o/\partial X_2^o)_{T,p,\gamma} \quad (6)$$

The composition of cholesterol at the interfacial region was calculated from the slopes of the $x_t^o$ against $X_2^o$ curves at fixed interfacial tension values. In Figure 2, the total concentration is plotted against the bulk composition at three representative fixed interfacial tension values and also against the calculated mixed film composition. These three pairs of curves are, in effect, equilibrium diagrams and explain how components are in equilibrium between the bulk phase and the absorbed film. They also show miscibility of components in the adsorbed film. In the top pair of curves, the compositions of the equilibrium expanded and condensed films are connected by a dotted line. Using this figure, we can learn the coexisting compositions of bulk and interface at any total concentration.

In Figure 3, the relation between the compositions of cholesterol at the interface and bulk phase is shown in the form of $X_2^H$ vs $X_2^o$ plot. It is observed that all the plots lie on the same curve within experimental error. It is worth

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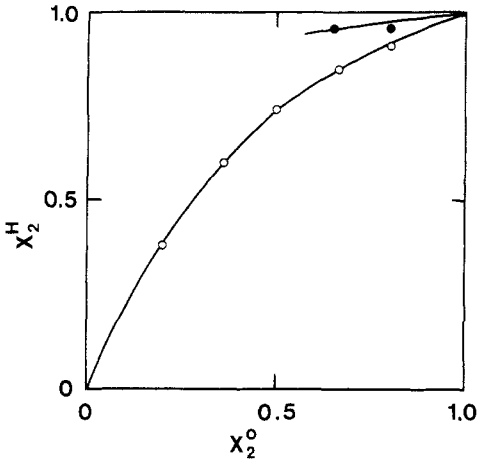


**Figure 3.** Relation between compositions in the oil and mixed absorbed phase. Open circles represent the expanded-state film; solid circles represent the condensed-state film.

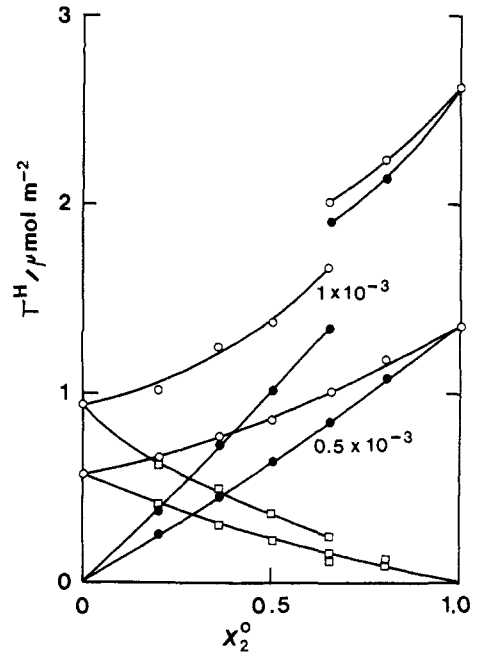


**Figure 4.** Variation of the interfacial densities with the bulk composition of cholesterol at the two fixed total concentrations. Open circle, solid circle, and square represent the total interfacial density, interfacial density of cholesterol, and interfacial density of octadecanol, respectively.

noting that the composition in the adsorbed film depends only on the bulk composition and state of the film. This fact suggests that the change in the interfacial density does not noticeably affect the composition of the mixed film. The composition of cholesterol in the expanded film is found to deviate to a larger value as compared to that in the oil phase. Further, the condensed film is said to be composed almost completely of cholesterol molecules.

Using the values of $X_2^H$ and $\Gamma_t^H$ obtained above, we evaluated the interfacial densities of octadecanol and cholesterol; they are plotted against bulk composition at two representative total concentrations and compared with the total interfacial density in Figure 4. Each of the total interfacial density curves shows a negative department from the straight line of the ideal mixing. If one expects the so-called condensing effect of the cholesterol molecules, the curve must be convex upward. Accordingly, we can say that the cholesterol molecules incorporated do not concentrate the mixed film. In contrast to an almost linear increase in the interfacial density of cholesterol, a somewhat steep decrease in that of octadecanol is observed. This implies that the increase in the number of cholesterol molecules causes the desorption of octadecanol molecules because of the strong lateral interaction between cholesterol molecules. At the total concentration of $1 \times 10^{-3}$, the phase transition takes place, changing from the expanded- to condensed-state film; the interfacial density of cholesterol increases, whereas that of octadecanol decreases.

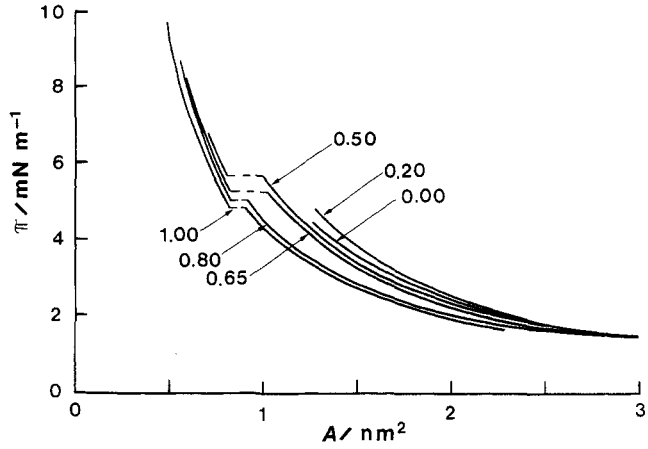


**Figure 5.** $\pi$–$A$ curve of the mixtures. The compositions of the cholesterol in the oil phase are shown.

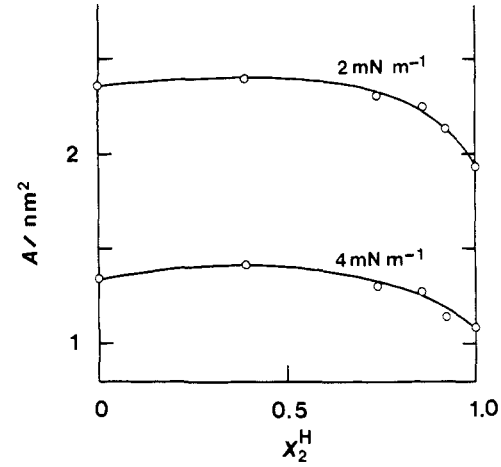


**Figure 6.** Variation of the mean area with the composition of cholesterol in the mixed film at two fixed interfacial pressures.

Suppose the monomolecular adsorption at the interface and the mean area per molecule, $A$, can be evaluated. The interfacial pressure $\pi$ defined by $\gamma^0 - \gamma$ ($\gamma^0$ being the interfacial tension of the pure carbon tetrachloride/water interface) is plotted against $A$ in Figure 5. The $\pi$–$A$ curve of octadecanol is almost the same as that at the hexane/water interface except for the phase transition. The cholesterol film is more condensed than the octadecanol film. The slope of the condensed cholesterol film is not as steep as that expected for the condensed film. It is yet uncertain whether it arises from impurities in the cholesterol used or peculiar properties of the cholesterol molecules adsorbed at the oil/water interface. The $\pi$–$A$ curves of mixed film lie between the pure components except for $X_2^o = 0.2$.

An interesting behavior of this mixed system is how the mean area varies with the incorporation of cholesterol into the interfacial film of octadecanol. Figure 6 shows the plots of the mean area against the composition of cholesterol in the film at two fixed interfacial pressure taken from Figure 5. Both curves, of which the states are expanded, ap-

parently exhibit a positive departure from the additive ideality of the area over the whole composition range.

The concept "condensing effect" is based on the experimental fact that the mean area of a mixed spread monolayer deviates negatively from the straight line joining those of the pure monolayers. However, monolayer studies suffer some disadvantages. Because of the strong lateral interaction of cholesterol molecules, the measurements can be made only for the condensed state of the cholesterol film. Since biological membranes are usually in a liquid-crystal state, the condensed-state film is inadequate to a model membrane to verify the condensing effect. In the condensed film, moreover, the cholesterol molecules have a limited miscibility with hydrocarbon chains.[6] This means that we have no method to obtain the straight line used commonly as a reference curve of the ideal mixing. In addition, the compressing process of the spread monolayer usually accompanies the hysteresis. Comparing the adsorbed film with the spread monolayer shows that the present study is free from the difficulties mentioned here.

The above experimental results do not conform with those of the condensing effect of cholesterol. If the variation of the mean area with the composition is assumed to be unaffected by carbon tetrachloride and water molecules in the film, the partial molar areas of the components are obtained from the intercepts of the tangent line on the coordinates. As can be seen from Figure 6, the partial molar area of octadecanol almost remains constant up to the composition of about 0.5 and then increases strongly with increasing composition. On the other hand, the partial molar area of cholesterol has a larger value than the molar area of the pure one and decreases to that with increasing composition. Such a variation in the partial molar area is attributable to the stronger cohesive force between cholesterol molecules than that between octadecanol and cholesterol molecules. Thus, we can conclude that the cholesterol molecule does not act as the condensing agent in a hydrocarbon chain region of membrane.

This conclusion is supported by the fact that the condensed film is composed almost completely of cholesterol (see Figures 2 and 3). It is also supported by the experimental work of Motomura et al.,[6] in which fatty acids do not mix with cholesterol in the condensed state and mix in a limited manner in the expanded state.

The partial miscibility of cholesterol in the condensed film corresponds to the phase separation of the phospholipid vesicle suspensions into cholesterol-rich and cholesterol-poor regions below the gel/liquid-crystalline phase transition temperature.[16-18]

Although cholesterol molecules are located so that their hydroxyl groups can be in the immediate vicinity of the phospholipid ester carbonyl groups,[19,20] the role of cholesterol molecules in biological membranes involves primarily the lateral interaction with fatty acyl chains. The effect of cholesterol on these acyl chains should be the same for any model membrane system. Thus, our conclusion conflicts with the current knowledge that cholesterol decreases the mean area per molecule occupied by saturated and monosaturated phospholipids in monolayers at the air/water interface.[3-5] Taking account of the disadvantages of spread monolayers, accordingly, the data on the spread monolayers must be analyzed with great caution.

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# Adsorption of Formaldehyde on Model MgO Surfaces: Evidence for the Cannizzaro Reaction

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Simultaneous formation of formate and methoxide species from formaldehyde on model MgO surfaces was observed with XPS and UPS. This reaction can be explained by a mechanistic route similar to the liquid-phase Cannizzaro reaction. The surface sites involved in this reaction are the base sites which are also responsible for the dissociation of methanol and water, as demonstrated by blocking experiments with these reagents. The difference between this reaction and the reaction of formaldehyde on other oxides to produce formates exclusively suggests that the operative pathways for consumption of hydrogen atoms eliminated from the carbonyl group may determine the reaction selectivity for formaldehyde conversion on oxide surfaces.

## Introduction

Surface formates and methoxides are two of the most ubiquitous reaction intermediates encountered in $C_1$ chemistry on solid surfaces. Formation of surface methoxides from methanol and of surface formates from formic acid, methanol, $CO/H_2$, and $CO/H_2O$ on metal and metal oxide surfaces has been reported in numerous adsorption studies.[1-12] Interconversion of these surface species also represents an important reaction sequence in certain

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