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Adsorption of Cholesterol at Hexane/Water Interface

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The interfacial tension between hexane solution of cholesterol and water was measured as a function of temperature and the mole fraction of cholesterol in hexane under atmospheric pressure. The interfacial tension vs. mole fraction of cholesterol curve was observed to have two break points at a certain temperature; the concentration of the break point increases with increasing temperature. The experimental results were analyzed by applying the rigorous thermodynamics of adsorption at interfaces developed previously. The excess number of moles of cholesterol and the thermodynamic quantity changes associated with its adsorption were found to show the discontinuous changes at the break points. It was concluded that the first-order phase transition takes place from the gaseous to the expanded state and from the expanded to the condensed one in the adsorbed film of cholesterol at the hexane/water interface.

Recently in our laboratory, the adsorbed films of long-chain aliphatic alcohol at oil/water interfaces have been proved to exhibit the frist-order phase transition from an expanded to a condensed state.[1-3] It is now interesting to see whether the phase transition takes place in the adsorbed film of surface-active substance having a rigid structure. Cholesterol is biologically important and its monolayers have been studied in some detail.[4,5] Furthermore the adsorbed film of cholesterol was found to display two types of phase transitions at the benzene/water interface.[6] Therefore, we are interested in examining the effect of the rigidity of cholesterol on the states of its adsorbed film at the oil/water interfaces and on the thermodynamic quantities of interface formation. In the present study, the adsorption of cholesterol at the hexane/water interface will be considered by measuring the interfacial tension as function of temperature and the mole fraction of cholesterol in the hexane phase. The results will be analyzed by use of the thermodynamics of interfaces.[7,8] Further, the thermodynamic quantity changes associated with the adsorption of cholesterol at the hexane/water interface evaluated will be compared with those of cholesterol at the benzene/water interface and those of 1-octadecanol at the hexane/water interface.

Experimental

Cholesterol (Sigma's standard for chromatography, 99+%) was used by without further purification. The purity was checked both thin-layer chromatography and gas-liquid chromatography. Hexane was refluxed over sodium and then distilled. Water distilled triply from alkaline permanganate solution. Their purities were checked by measuring the equilibrium interfacial tension between them.

The interfacial tension was measured by the pendant drop technique method within a precision of $0.05\,\mathrm{mN\,m^{-1}}$. Further description of the apparatus and method was given previously.[9,10] Temperature was held constant within 0.05 K.

Results and Discussion

Figure 1 shows the dependence of the interfacial tension γ between hexane solution of cholesterol and water on temperature T at various mole frations x_1 under atmospheric pressure. From this figure, we can see that there are five types of the curves. First the γ value at a very low concentration decreases gradually with increasing T in a similar manner to that of the pure hexane/water interface. Second, at a relatively low concentraration, the γ vs. T curve has one break point. The third type of the γ vs. T curve is characterized by two break points; the three regions have different slopes. At a relatively high concentra-

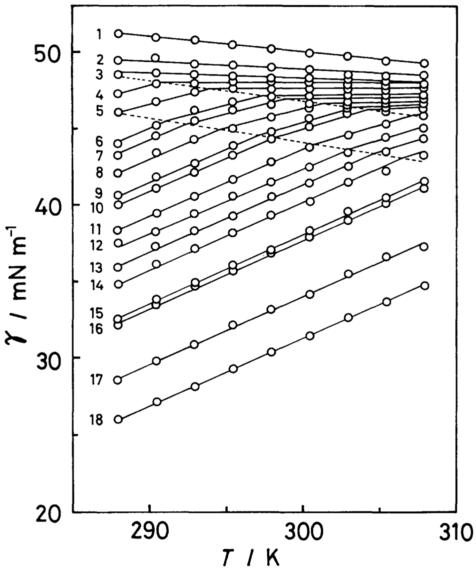

Fig. 1. Interfacial tension vs. temperature curves at constant mole fraction under atmospheric pressure. (1): $10^4x_1=0$, (2): 0.17, (3): 0.29, (4): 0.45, (5): 0.53, (6): 0.66, (7): 0.70, (8): 0.77, (9): 0.91, (10): 1.03, (11): 1.25, (12): 1.34, (13): 1.54, (14): 1.75, (15): 2.02, (16): 2.15, (17): 3.02, (18): 3.90.

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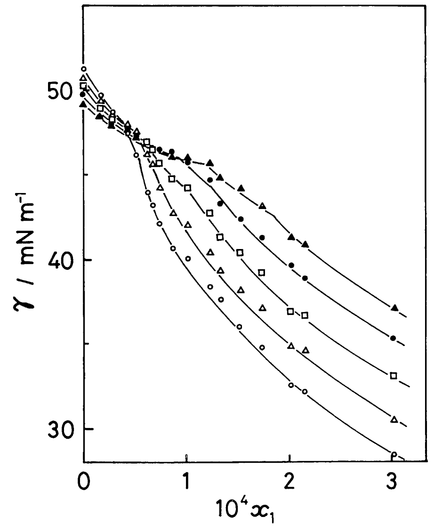

Fig. 2. Interfacial tension vs. mole fraction curves at constant temperature under atmospheric pressure. (○): 288.15 K, (△): 293.15 K, (□): 298.15 K, (●): 303.15 K, (▲): 308.15 K.

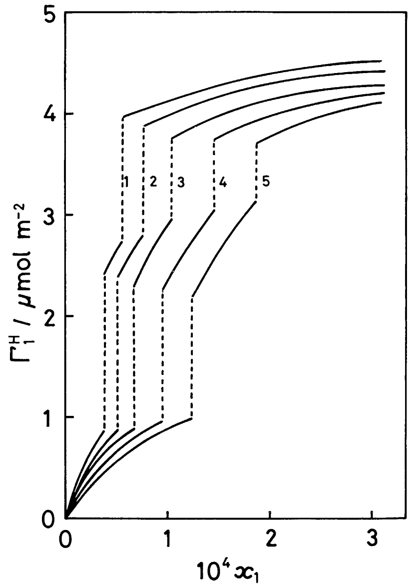

Fig. 3. Interfacial excess number of moles vs. mole fraction curves at constant temperature under atmospheric pressure. (1): 288.15 K, (2): 293.15 K, (3): 298.15 K, (4): 303.15 K, (5): 308.15 K.

tion, one of the break points on the curve disappears; this curve is classified as the forth type. Finally, at a high concentration, the γ value increases linearly with increasing T. This behavior is slightly different from that of cholesterol at the benzene/water interface at the point that the break points of the latter are vague.[6] Shown in Fig. 2 are the γ vs. x_1 curves at several temperatures under atmospheric pressure depicted by picking up the values of γ from Fig. 1; γ vs. x_1 curve definitely has two break points corresponding to the ones on the γ vs. T curves observed in Fig. 1. In a concentration range beyond the concentration of the second break point, the γ value decreases rapidly with increasing x_1.

It is reasonably assumed for the system that the mutual solubility of hexane and water is negligible, cholesterol is insoluble in water, and the hexane solution of cholesterol behaves ideally. According to the rigorous thermodynamics of adsorption at interfaces,[7,8] the interfacial excess number of moles of cholesterol can be calculated by applying

$$\Gamma_1^{\mathrm{H}} = -(x_1/RT)(\partial\gamma/\partial x_1)_{T,p} \qquad (1)$$

to the γ vs. x_1 curve at constant T and p given in Fig. 2. The Γ_1^{H} vs. x_1 curves obtained at various temperatures under atmospheric pressure are shown in Fig. 3. The value of Γ_1^{H} is seen to increase steeply with increasing x_1 even at a low concentration of the order of 10^{-5} in x_1 and changes discontinuously at the concentrations corresponding the break points. At a concentration above the second break point, the value of Γ_1^{H} is found to be little affected by the concentration. The discontinuous changes clearly indicate that two kinds of phase transitions take place in the adsorbed film. Furthermore, the Γ_1^{H} value and its difference at the phase transition point are found to decrease gradually as the temperature increases.

In order to make sure what is the state of the adsorbed film, it is advantageous to draw the interfacial pressure π vs. area per molecule A curve, π and A being defined, respectively, as

$$\pi = \gamma^0 - \gamma \qquad (2)$$

and

$$A = 1/\Gamma_1^{\mathrm{H}} N_{\mathrm{A}}, \qquad (3)$$

where γ^0 is the interfacial tension of pure hexane/water interface and N_{A} the Avogadro's number. By use of the γ vs. x_1 curves in Fig. 2 and the values of Γ_1^{H} in Fig. 3, the π vs. A curves were obtained at various temperatures under atmospheric pressure; they are depicted together with those of cholesterol at the benzene/water interface[6] and of 1-octadecanol at the hexane/water interface[1] at 298.15 K in Fig. 4. All the curves are seen to show two discrete changes in A at the transition pressures. On the other hand, it has been known that the insoluble monolayer of cholesterol at the air/water interface forms a condensed film with the area per molecule of about 0.4 nm², in which cholesterol molecules are packed closely and oriented vertically.[4,5] Comparing these results, we can conclude that the two break points on the γ vs. x_1 curve exhibit the first-order phase transitions from the gaseous to the expanded film and from the expanded to the condensed one. Further we find from Fig. 4 that the area of cholesterol molecule in the condensed state

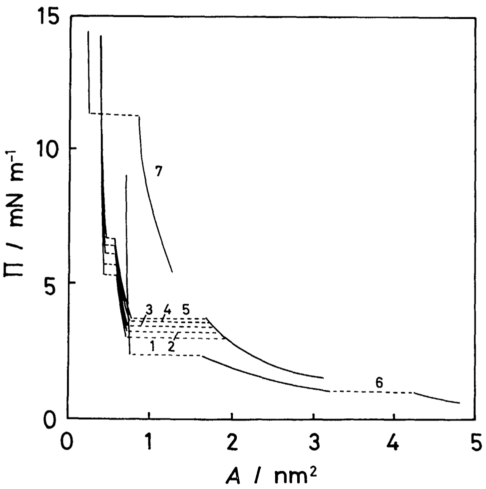

Fig. 4. Interfacial pressure vs. area curves at constant temperature under atmospheric pressure. (1): 288.15 K, (2): 293.15 K, (3): 298.15 K, (4): 303.15 K, (5): 308.15 K, (6): cholesterol at benzene/water interface, 298.15 K, (7): 1-octadecanol at hexane/water interface, 298.15 K.

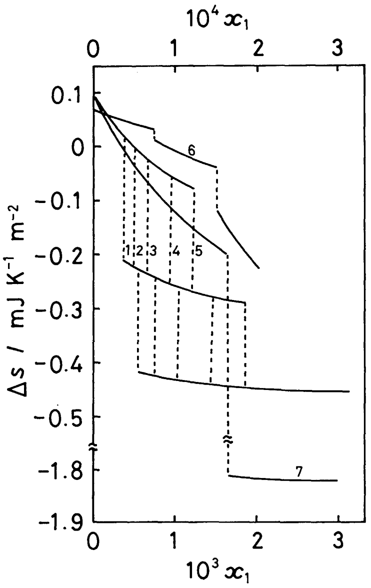

Fig. 5. Entropy change vs. mole fraction curves of cholesterol at constant temperature under atmospheric pressure. (1): 288.15 K, (2): 293.15 K, (3): 298.15 K, (4): 303.15 K, (5): 308.15 K, (6): cholesterol at benzene/water interface at 298.15 K, (7): 1-octadecanol at hexane/water interface at 298.15 K.

is about twice that of 1-octadecanol molecule. This finding is explicable by the large size and rigid structure of cholesterol molecule. Moreover the area of cholesterol at the benzene/water interface is observed to be more expanded when compared with that of cholesterol at the hexane/water interface.

In order to clarify the adsorption behavior of cholesterol at the interface in further detail, it is advantageous to evaluate and examine the thermodynamic quantities of interface formation. At first, the entropy change associated with the adsorption of cholesterol Δs can be evaluated by

$$\Delta s = -(\partial \gamma / \partial T)_{p,x_1}. \tag{4}$$

Figure 5 demonstrates the Δs vs. x_1 curves at various temperatures under atmospheric pressure obtained by applying Eq. 4 to the γ vs. T curves in Fig. 1. The Δs value seems to be independent of temperature whereas the concentration of the phase transition depends appreciably on temperature. Inspecting the variation in Δs with x_1, we notice that the Δs values of the gaseous and expanded films decrease gradually with increasing x_1, while the one of the condensed film does not change appreciably with x_1. Comparing Fig. 5 with Fig. 3, we can conclude that the value of Δs is correlated to that of Γ_1^{H} and the adsorption of cholesterol makes a negative contribution to Δs. On the other hand, as seen in Fig. 5, the value of Δs at the benzene/water interface decreases even in the case of the condensed film. This fact indicates that cholesterol forms a loose film at the benzen/water interface. Now the comparison of the entropy change of cholesterol with the corresponding one of 1-octadecanol seems to be useful. The Δs vs. x_1 plots of 1-octadecanol at 298.15 K under atmospheric pressure is also shown in Fig. 5. It is apparent that the phase transition of 1-octadecanol from the expanded to the condensed state is attended by a large decrease in entropy while that of cholesterol is done by a small decrease. Accordingly, in the state of the condensed film, the absolute value of Δs of cholesterol is considerably small compared with that of 1-octadecanol. Such a behavior is attributable to the rigid structure of cholesterol; that is, the conformation of cholesterol is not so much changed when the molecule is transferred from the hexane solution to the adsorbed film.

Let us now verify thermodynamically that the break point on the γ vs. T curve represents the occurence of the phase transition. The two states, say α and β, of the adsorbed film are described, respectively, by

$$\mathrm{d}\gamma = -\Delta s^{\alpha}\mathrm{d}T + \Delta v^{\alpha}\mathrm{d}p - \Gamma_1^{\mathrm{H},\alpha}(\partial \mu_1/\partial x_1)\mathrm{d}x_1 \tag{5}$$

and

$$\mathrm{d}\gamma = -\Delta s^{\beta}\mathrm{d}T + \Delta v^{\beta}\mathrm{d}p - \Gamma_1^{\mathrm{H},\beta}(\partial \mu_1/\partial x_1)\mathrm{d}x_1. \tag{6}$$

Since the two phases are in equilibrium, elimination of the variable x_1 between Eqs. 5 and 6 at constant pressure and rearrangement of the resulting equation yield the following equation;

$$(\partial \gamma^{\mathrm{eq}}/\partial T)_p = -(\Delta s^{\beta}/\Gamma_1^{\mathrm{H},\beta} - \Delta s^{\alpha}/\Gamma_1^{\mathrm{H},\alpha}) / (1/\Gamma_1^{\mathrm{H},\beta} - 1/\Gamma_1^{\mathrm{H},\alpha}) \tag{7}$$

where γ^{eq} is the equilibrium interfacial tension. The left side of Eq. 7 is estimated from the slope of the

Table 1. Comparison between Left and Right Sides of Eq. 7 at 298.15 K under Atmospheric Pressure

Gaseous/Expanded		Expanded/Condensed	
Eq. 7/mJ K^{-1} m^{-2}		Eq. 7/mJ K^{-1} m^{-2}	
ls.	rs.	ls.	rs.
−0.13	−0.10	−0.16	−0.31

dotted curve depicted in Fig. 1 and the right side is calculated by use of the quantities shown in Figs. 3 and 5, so that we can check whether Eq. 7 holds for the present system. The values of the left and right sides of Eq. 7 were evaluated for the phase transitions and compared with each other in Table 1. Taking account of errors in calculations, the agreement between both sides of the equation is not unsatisfactory. Consequently, we can say that the adsorbed film of cholesterol exhibits the first-order phase transition from a gaseous to an expanded film and that from an expanded to a condensed film.

Finally, the energy change associated with the adsorption Δu can be calculated by making use of the relation

$$\Delta u = \gamma + T\Delta s - p\Delta v. \tag{8}$$

The numerical values of the first and second terms on the right side are obtained from Figs. 2 and 5, respectively. The third term was proved to be negligibly small compared with the other terms under atmospheric pressure.[1] The Δu value evaluated is plotted against x_1 at 298.15 K under atmospheric pressure in Fig. 6. In the gaseous and expanded states, the value of Δu decrease gradually with an increase in concentration, while in the condensed state it is almost independent of the concentration. Taking into account that the Γ_1^{H} value is nearly constant in the condensed state, this finding suggests that the adsorption of cholesterol at the interface makes a negative contribution to the energy change. Consequently, we can say that the adsorption of cholesterol diminishes the energy of the hexane/water interface and overcomes the disadvantage caused by the decrease in entropy.

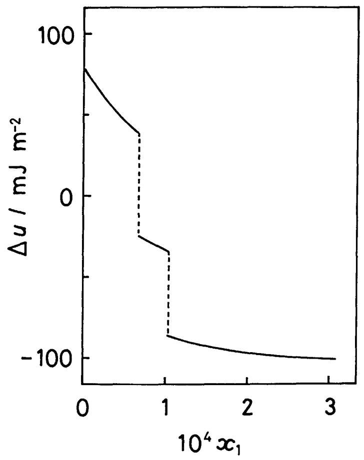

Fig. 6. Energy change vs. mole fraction curve at 298.15 K under atmospheric pressure.

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