Formation and Collapse of Mixed Monolayers of Trilaurin with Cholesterol and with Tristearin[1)]

Masayuki NAKAGAKI and Noriaki FUNASAKI*
Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606

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The surface pressure-area curves of mixed monolayers and the equilibrium spreading pressure of solid mixtures were measured at 25 °C for cholesterol and trilaurin as well as tristearin and trilaurin. The phase diagrams of these mixtures were obtained by the measurement of the melting point. From two curves, one representing the relation between the collapse pressure (if the mixed monolayer was not overcompressed) and the composition of mixed monolayer, and the other representing the relation between the equilibrium spreading pressure and the composition of solid mixture, it was shown that the compositions of the mixed monolayer and the solid mixture coexisting in equilibrium were obtained. It was found that when the mixed monolayer of cholesterol and trilaurin was compressed, pure trilaurin began to collapse and deposit, and that the mixed monolayer of tristearin and trilaurin was apt to be overcompressed. It was concluded on the basis of phase rule and thermodynamics for plane interfaces that the equilibrium spreading pressure is constant regardless of the composition of solid mixture if the solid mixture consists of two phases of mixed crystals, and that cholesterol and trilaurin form a regular solution in mixed monolayer.

The miscibility in a mixed monolayer can be discussed on the basis of the composition dependence of collapse pressure of the mixed monolayer.[2–4)] A theoretical study has recently been reported on the equilibrium spreading pressure of mixed crystals.[5)] In both processes, *i.e.*, when a lipid mixture forms a monolayer and when a mixed monolayer collapses and deposits a lipid mixture, a mixed monolayer and a lipid mixture coexist on the air-water interface. In order to clarify the coexisting state of a mixed monolayer and a bulk mixture, the phase rule[6)] and thermodynamics of plane interface seem to be useful. However, a thorough study has not been reported yet.

In the present investigation, the collapse pressure of mixed monolayers of cholesterol and trilaurin as well as tristearin and trilaurin and also the equilibrium spreading pressure of these solid mixtures were measured and these results were discussed on the basis of the phase rule and thermodynamics of plane interface.

Experimental

Materials. Trilaurin and tristearin used in this work were the same as those used previously.[7)] They were synthesized directly from the corresponding fatty acid chloride and glycerol and then purified by repeated recrystallization. Cholesterol (guaranteed grade, Wako Pure Chemicals, Ltd.) was recrystallized from ethanol.[8)]

Measurement of Surface Pressure. The surface pressure-area curve of mixed monolayers of cholesterol and trilaurin as well as tristearin and trilaurin were drawn by measuring the surface pressure of the monolayer which was compressed at nearly constant speed.

Solid mixtures of cholesterol and trilaurin as well as tristearin and trilaurin were prepared by mixing the chloroform solutions of both components and evaporating the solvent gradually at room temperature. When this crystalline mixture was sprinkled gently onto a clean aqueous surface, the surface pressure increased and reached a constant value. When this was compressed to some extent and then kept at that area, the surface pressure once increased and then decreased to a value nearly equal to the original value. By averaging these two equilibrium values of surface pressure, the equilibrium spreading pressure, F_e, was determined. Although a part of the solid mixture spreads as a monolayer, the composition of the solid mixture does not vary, since the amount of mixture sprinkled was more than, at least, a hundred times as much as required to form the monolayer. The surface pressure was measured at 25±0.1 °C. Details of the measurement of surface pressure have been reported.[7)]

Measurement of Melting Point. The solid was mixed in the same way as described above. The initial and final melting points of the solid mixture were measured with a Yanagimoto micro-melting point apparatus, SR-50, equipped with a polarization microscope.

Results

Surface Pressure-Area Curve. The relations between surface pressure F and mean area A of mixed monolayers of trilaurin with cholesterol and with tristearin are shown in Figs. 1 and 2, respectively. The kink-points (indicated by arrows) are the points at which the monolayers begin to collapse. The pressure and area

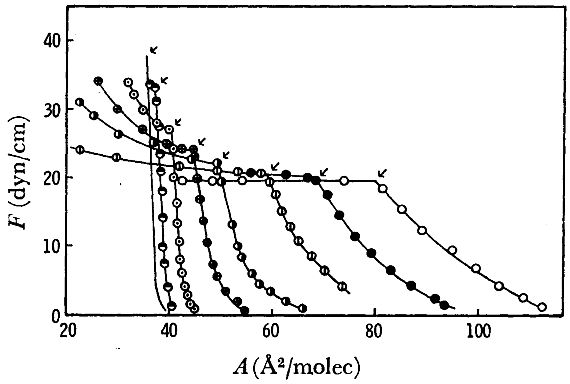

Fig. 1. The surface pressure-mean area curves of mixed monolayers of cholesterol and trilaurin of various mole ratios.
Cholesterol: trilaurin
—: 1: 0, ◓: 9: 1, ⊙: 4: 1, ⊕: 2: 1, ◑: 1: 1, ⦶: 1: 2, ●: 1: 5, ○: 0: 1.

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* Present address: *Kyoto College of Pharmacy, 5-Nakauchi-cho, Misasagi Yamashina, Higashiyama-ku, Kyoto 607.*

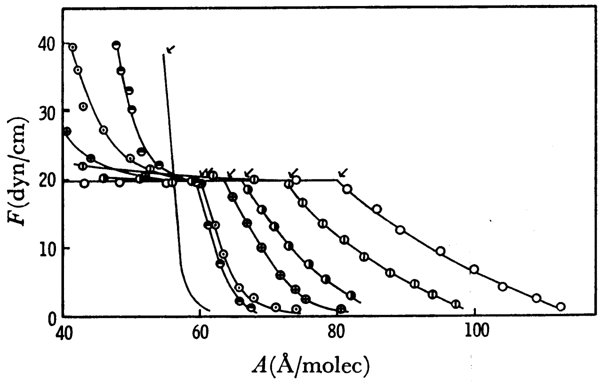

Fig. 2. The surface pressure-mean area curves of mixed monolayers of tristearin and trilaurin of various mole ratios.
Tristearin: trilaurin
—: 1: 0, ◓: 9: 1, ⊙: 4: 1, ⊕: 2: 1, ◑: 1: 1, ⦶: 1: 2, ○: 0: 1.

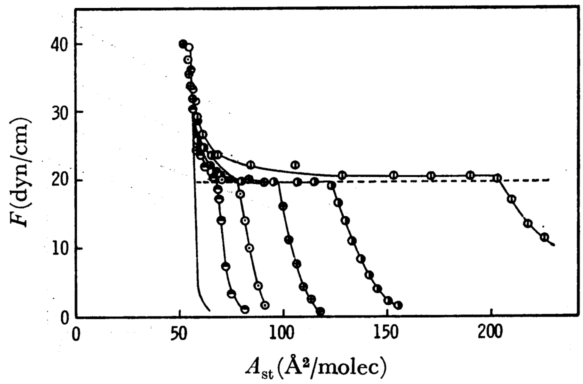

Fig. 4. The relation between surface pressure and area per molecule of tristearin for mixed monolayers of tristearin and trilaurin of various mole ratios.
Tristearin: trilaurin
—: 1: 0, ◓: 9: 1, ⊙: 4: 1, ⊕: 2; 1, ◑: 1: 1, ⦶: 1: 2.
------: Collapse pressure of trilaurin.

of these points are collapse pressure F_c and collapse area A_c, respectively. The deposit formed by the collapse of a monolayer is expected to contain a component with lower collapse pressure in larger amount than one with a higher collapse pressure. In an extreme case, it is possible that only the former comes into the deposit, the latter remaining entirely in the monolayer. This might be clarified by dividing the area by the number of molecules of the component whose collapse pressure is higher, instead of the total number of initially spread molecules in the mixed monolayer, and by comparing the area thus obtained with the area per molecule in the pure monolayer of the component of higher collapse pressure. We see from Figs. 1 and 2 that the component with lower collapse pressure is trilaurin in both mixed monolayers. Instead of the mean area A, the area occupied by one molecule of cholesterol A_{ch}, and that occupied by one molecule of tristearin A_{st}, are plotted in Figs. 3 and 4, respectively. All the experimental values lies on one and the same curve (dotted line) at a pressure higher than collapse pressure regardless of the composition initially spread (Fig. 3). On the other hand, the collapse pressure is the same and equal to that of a trilaurin monolayer (dotted line) but the area at higher pressure varies with initial composition (Fig. 4).

Relation between Mean Area and Mole Fraction in the Mixed Monolayers. The relations between the mean area A at a given pressure and the mole fraction of trilaurin in the mixed monolayers x_l^s are given in Figs. 5 and 6, respectively. In these figures, the dotted line is calculated by the equation

$$A = x_1^s A_1 + x_2^s A_2 \qquad (1)$$

where x^s is the mole fraction in a monolayer, A the mean area per molecule, and subscripts 1 and 2 indicate the components. The experimental values of the area of mixed monolayer of tristearin and trilaurin agree with the values from Eq. (1) (Fig. 6), while the experimental values are smaller than the calculated values for the monolayer of cholesterol and trilaurin (Fig. 5).

Equilibrium Spreading Pressure and Collapse Pressure. Equilibrium spreading pressure was measured by gently sprinkling a large quantity of the crystalline mixture on the water surface. In the case of the mixture of tri-

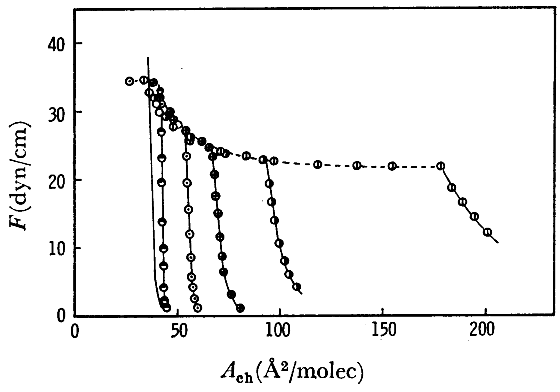

Fig. 3. The relation between surface pressure and area per molecule of cholesterol for mixed monolayers of cholesterol and trilaurin of various mole ratios.
Cholesterol: trilaurin
—: 1: 0, ◓: 9: 1, ⊙: 4: 1, ⊕: 2: 1, ◑: 1: 1, ⦶: 1: 2.

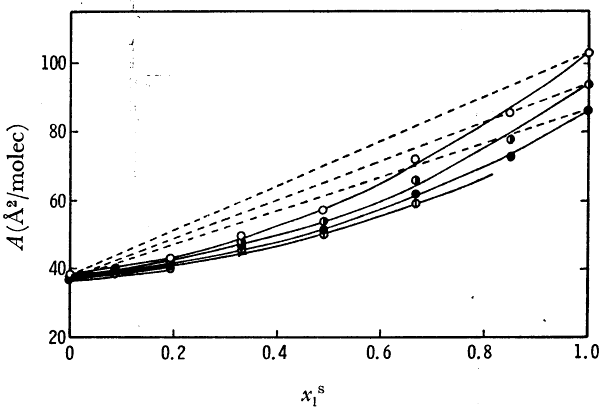

Fig. 5. The relation between mean area and mole fraction of trilaurin for mixed monolayers of cholesterol and trilaurin at various surface pressures.
------: Line calculated from Eq. (1).
F(dyn/cm)
○: 5, ◑: 10, ●: 15, ⦶: 20, ◓: 27.5.

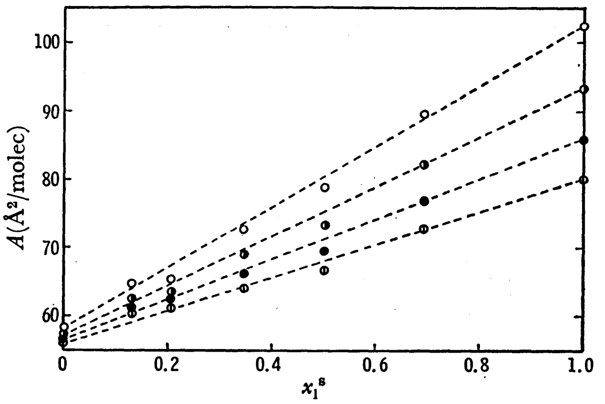

Fig. 6. The relation between mean area and mole fraction of trilaurin for mixed monolayers of tristearin and trilaurin at various surface pressures.
------: Line calculated from Eq. (1).
F(dyn/cm)
○: 5, ◑: 10, ●: 15, ⦶: F_c.

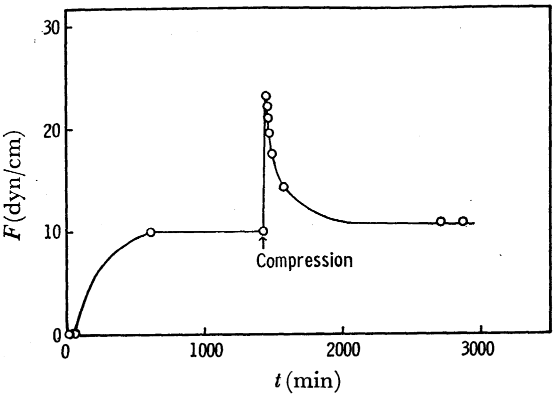

Fig. 7. Equilibrium spreading pressure of solid mixture of tristearin and trilaurin (1: 1).

stearin and trilaurin at the molar ratio of 1: 1, the surface pressure increased and reached a constant value (Fig. 7). When the monolayer in this state was compressed to some extent at the time indicated by an arrow, the pressure increased rapidly and returned gradually to the original value. Equilibrium spreading pressure was determined as the mean value. It was thus confirmed that the crystal and the monolayer were in equilibrium. It took about one minute for trilaurin, about 30 min for cholesterol and more than 10 day for tristearin to return nearly to the original value after the monolayer had been compressed and kept at that area, while in mixed monolayer, it took about 30 min for the cholesterol–trilaurin system.

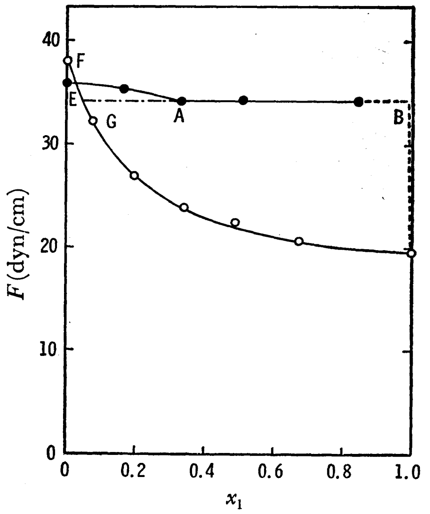

Fig. 8. Equilibrium spreading pressure and collapse pressure for mixed system of cholesterol and trilaurin.
●: $F_e-x_l^b$, ○: $F_c-x_l^s$.

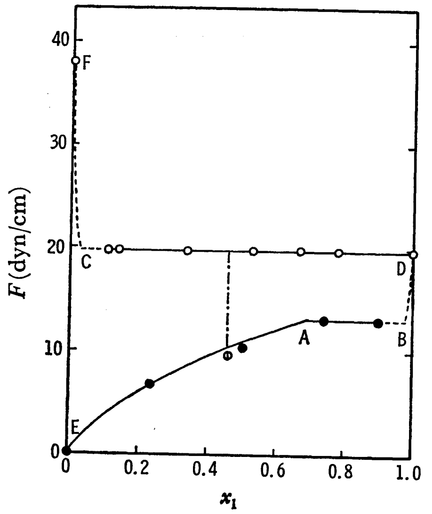

Fig. 9. Equilibrium spreading pressure and collapse pressure for mixed system of tristearin and trilaurin.
●: $F_e-x_l^b$, ○: $F_c-x_l^s$.

The relation between the equilibrium spreading pressure and mole fraction of trilaurin in solid mixture is shown by closed circles for cholesterol and trilaurin in Fig. 8 and for tristearin and trilaurin in Fig. 9. The composition of point B was uncertain, and the equilibrium spreading pressure near this point (shown by dotted lines) was not measured. The relation between the collapse pressure F_c, obtained from Figs. 1 and 2, and the mole fraction of trilaurin in the mixed monolayers x_l^s is also shown by open circles in Figs. 8

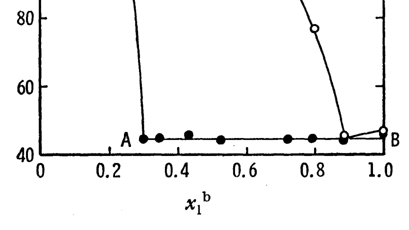

Fig. 10. The relation between melting points and mole fraction of trilaurin for a mixture of cholesterol and trilaurin.
●: Initial melting point, ○: Final melting point.

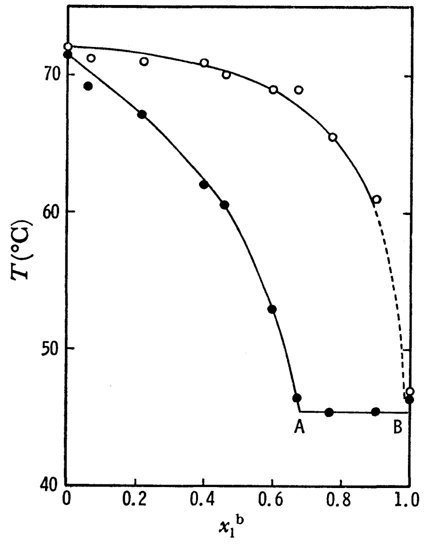

Fig. 11. The relation between melting points and mole fraction of trilaurin for a mixture of tristearin and trilaurin.
●: Initial melting point, ○: Final melting point.

and 9, respectively.

Melting Point of Solid Mixture. The melting points of solid mixtures for cholesterol and trilaurin and for tristearin and trilaurin are shown in Figs. 10 and11, respectively. Closed circles indicate the initial melting points and open circles the final melting points.

Discussion

Pressure-Area Curve. A_{ch} of mixed monolayer is larger than the area occupied by one molecule of cholesterol in its own monolayer (Fig. 3). The pressure-area curves of mixed monolayers at higher pressure than collapse pressure are on a single line regardless of the composition of initially spread monolayer. These two facts indicate that when the mixed monolayer collapses, only trilaurin deposits to solid and all the initially spread molecules of cholesterol remain in the monolayer, containing a part of trilaurin, and that the molar ratio of trilaurin to cholesterol in the monolayer depends only on the surface pressure regardless of the composition of the initially spread monolayer.

The mixed monolayer begins to collapse at the pressure equal to the collapse pressure of the trilaurin monolayer and after all the trilaurin molecules have deposited, *viz.*, the area A_{st} has reached the occupied area of the tristearin monolayer, the monolayer collapses again at the collapse pressure of the pure tristearin monolayer (Fig. 4). At a pressure lower than the collapse pressure, the mean area of the mixed monolayer agrees with the additivity of the area given by Eq. (1) (Fig. 6). Thus, it might be concluded that tristearin and trilaurin are completely immiscible in monolayer. However, the fact that the F-A_{st} curve varies with the composition of initially spread monolayer in the range from the first collapse pressure (equal to that of trilaurin monolayer) to the second collapse pressure (equal to that of tristearin monolayer), cannot be explained without taking the interaction between both components into account.

Equilibrium Spreading Pressure and Collapse Pressure. A phase diagram of cholesterol–trilaurin system is given in Fig. 8. The mixed monolayer exists at a surface pressure lower than the curve of collapse pressure (open circles), but mixed monolayer and solid coexist in the range between this curve and the curve of equilibrium spreading pressure (closed circles). If a horizontal line is drawn at a given surface pressure, its intersection with the curves of equilibrium spreading pressure and collapse pressure give mole fractions of trilaurin in the solid and in the monolayer, respectively, coexisting on the water surface. In this meaning, the curve of collapse pressure and that of equilibrium spreading pressure might be called monolayer and solidus lines, respectively, the latter being more generally called bulk phase line. It is seen that cholesterol, whose equilibrium spreading pressure is higher, is richer in the monolayer and trilaurin, whose equilibrium spreading pressure is lower, is richer in the solid phase. The mixed monolayers (Fig. 3) were those whose mole fraction of trilaurin was greater than x_l^G (Fig. 8), *viz.*, the mole fraction 0.06 of trilaurin at point G. In this range of composition, nearly pure trilaurin is expected to deposit, when the mixed monolayer is compressed. This agrees with the result given in Fig. 3. As expected, when the mixed monolayer was further compressed, the surface pressure increased and remained constant at about 34.2 dyn/cm, not increasing with further compression (Fig. 3). Further compression was not carried out, since the mixed monolayer had spread over the top of the trough.

The equilibrium spreading pressure is constant over the range of composition between A and B in Fig. 8. For interpretation, the phase rule for plane interface might be used. The number of freedom, ω, is given by6)

$$\omega = c - \varphi - (\phi - s) + 2 \qquad (2)$$

where c is the number of components, φ is the number of the bulk phase, ϕ is the number of interface phases and s is the number of types of interface. If the solid deposits over the range of composition between x^A and x^B is a mixture of two mixed crystals S^A, and S^B, whose compositions are x^A and x^B, respectively, and if A and W denote air and water, respectively, then $c=4$ (A, W, cholesterol and trilaurin), $\varphi=4$ (A, W, S^A and S^B) $s=6$ (A/W, A/S^A, A/S^B, W/S^A, W/S^B and S^A/S^B), and $\phi=6$ (one phase at each interface). Thus we get $\omega=2$. Since temperature and pressure are kept constant, the surface pressure and composition of mixed monolayer should be constant irrespective of the composition of the solid mixture. The equilibrium spreading pressure is thus found to be constant in the range of composition between A and B in Fig. 8. Comparing this with the phase diagram of the bulk phase, it is found from Fig. 10 that the solids of the compositions of x^A and x^B coexist at 44.5 °C. If these compositions are considered nearly unvaried at temperatures below 44.5 °C, they correspond to compositions A and B (25 °C).

In the case of the tristearin–trilaurin system, the components were suspected of being immiscible in the mixed monolayer (Figs. 4 and 6). However, it is not certain whether the monolayer is in equilibrium with the solid formed when the monolayer collapses, or not. The collapse pressure of tristearin monolayer is 38 dyn/cm, while the equilibrium spreading pressure is 0 dyn/cm (Fig. 9). This discrepancy may be due to the fact that the rate of collapse of the tristearin monolayer is low, so that its monolayer is apt to be overcompressed. Actually, the surface pressure decreased gradually with time and reached 1 dyn/cm after ten days, when the area of the tristearin monolayer was kept constant as soon as the monolayer began to collapse. Thus, the mixed monolayer of tristearin and trilaurin, also, has the possibility of being overcompressed. If the area of the mixed monolayer of tristearin and trilaurin at $x_l=0.406$ was kept constant as soon as the monolayer began to collapse, the surface pressure decreased (Fig. 9) down to the value ① after two days and did not decreased any more. The collapse pressure obtained from the pressure-area curves of the mixed monolayer of tristearin and trilaurin (Figs. 2 and 4) is too great to be in an equilibrium state. It cannot be concluded from the results (Figs. 4 and 6) that tristearin and trilaurin are immiscible in the mixed monolayer.

Since tristearin and trilaurin are completely miscible in the liquid state and partially miscible in the solid state (Fig. 11), it is unlikely that they are completely immiscible in the monolayer state. Therefore, the result showing the establishment of the additivity of the area (Fig. 6) should be interpreted as the ideal mixing in the monolayer.

The equilibrium spreading pressure is constant over the range from x_l^A to x_l^B (Fig. 9). This is interpreted to be due to the coexistence of the mixed crystals of the compositions x_l^A and x_l^B, corresponding to points A and B (Fig. 8) for the mixture of cholesterol and trilaurin. It is found that the compositions of points A and B (Fig. 9) are equal to those of points A and B (Fig. 11).

Thermodyndmic Treatment of the Equilibrium between Monolayer and Solid. Only trilaurin is deposited as a solid and the mixed monolayer is in equilibrium with the pure solid of trilaurin when the mixed monolayer of cholesterol and trilaurin collapses. Since the chemical potential of trilaurin in the mixed monolayer is equal to that in the solid, the following equation can be written according to Joos.[9]

$$\mu_l^{ob} = \mu_l^{os} + kT\ln\gamma_l^s x_l^s + F\bar{A}_l \tag{3}$$

where μ^0 is the standard chemical potential, γ the activity coefficient, x the mole fraction and $\bar{A}$ the partial molecular area. Superscripts b and s indicate a solid and a monolayer, respectively. For one-component system of trilaurin, we have

$$\mu_l^{ob} = \mu_l^{os} + F_{lc}A_{lc} \tag{4}$$

with $x_l^s=1$, $\bar{A}_l=A_{lc}$ and $\gamma_l^s=1$. Substituting this equation into Eq. (3), we obtain the following equation for the surface activity coefficient of trilaurin γ_l^s.

$$\gamma_l^s = \frac{1}{x_l^s}\exp\left(\frac{F_{lc}A_{lc}-F\bar{A}_l}{kT}\right) \tag{5}$$

From Fig. 1 we have $F_{lc}=19.4$ dyn/cm, and $A_{lc}=80.5$ Å^2/molec. The mole fraction of trilaurin in the mixed monolayer x_l^s at a given surface pressure is obtained from the curve of collapse pressure (Fig. 8). The value of $\bar{A}_l$ is obtained from the intercept of the tangent at the composition of x_l^s on the curve representing the relation between the mean area A and the mole fraction of the monolayer x_l^s under the given pressure. The right hand of Eq. (5) can be calculated at a given surface pressure. Proportionality holds between the logarithm of the surface activity coefficient of trilaurin γ_l^s and the square of the mole fraction of cholesterol in the monolayer $(1-x_l^s)^2$ (Fig. 12). If we denote proportionality constant by B/kT, we have

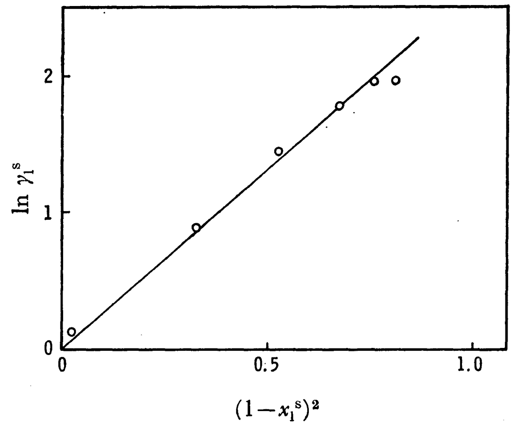

Fig. 12. The relation between the logarithm of surface activity coefficient of trilaurin and the square of mole fraction of cholesterol in mixed monolayer of cholesterol and trilaurin.

$$\ln\gamma_l^s = \frac{B}{kT}(1-x_l^s)^2. \tag{6}$$

$B=1.58$ kcal/mol is obtained from Fig. 12. Equation (6) is the relationship expected from the theory of regular solution. Thus, it is concluded that cholesterol and trilaurin form a regular solution in the monolayer.

References

1) Presented at the 24th Colloquium on Colloid and Interface Chemistry, Gifu, October, 16, 1971.

2) D. J. Crisp, "Surface Chemistry," Supplement to Research, Butterworths, London (1949), p. 23.

3) G. L. Gaines, "Insoluble Monolayers at Liquid-Gas Interface," Interscience Publishers (1966), p. 281.

4) M. C. Phillips and P. Joos, *Kolloid-Z. Z. Polym.*, **238**, 499 (1970).

5) P. Joos, *J. Colloid Interfac. Sci.*, **35**, 215 (1971).

6) R. Defay, I. Prigogine, A. Bellemans, and D. H. Everett, "Surface Tension and Adsorption," Longmans, London (1966), p. 77.

7) M. Nakagaki, N. Funasaki, and K. Fujita, *Nippon Kagaku Zasshi*, **93**, 243 (1972).

8) M. Nakagaki and N. Funasaki, *ibid.*, **1972**, 1576.

9) P. Joos, *Bull. Soc. Chim. Belges*, **78**, 207 (1969).