The Penetration of Disodium Hexadecyl Phosphate to the Monolayers of Cholesterol and its Esters from the Underlyning Aqueous Solutions

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The surface pressure of the monolayers of cholesterol (CH), cholesteryl acetate(CA), and cholesteryl propionate (CP) on the aqueous solutions of disodium hexadecyl phosphate (SHP) was measured. The monolayer exhibited a more expanded state on the solution than on pure water. The curves showing the surface pressure against the area per molecule of each monolayer had a kink point, which was considered to indicate the collapse of the monolayer. The amount of SHP penetrating into the insoluble monolayer was calculated by means of the Gibbs adsorption equation. It was found that the relation between the penetration amount and the activity of SHP in the aqueous solution could be expressed by a modification of the Langmuir adsorption equation. It was also found that the saturated amount of the penetration decreased, and the Langmuir coefficient increased, with the compression of the monolayer.

To study the penetration of a physiologically active substances from its aqueous solution into an insoluble lipid monolayer is important and interesting. Many papers on this subject have been published on, *e.g.*, the penetration of surfactants into the cholesterol or long-chain alcohol monolayers,[1-3] the penetration of anesthetics into the phospholipid monolayers,[4] and the penetration of various proteins into the phospholipid monolayers.[5-7] These papers have presented valuable discussion related to the fundamental aspect of the drug action, the biomembrane structure, the interaction of antibodies with lipid antigens, the enzyme activation, and so forth.

When an insoluble monolayer is spread on an aqueous solution of a surface-active substance, the latter penetrates into the insoluble monolayer and a mixed monolayer of these components is formed. In our laboratory, the thermodynamics of mixed monolayers of insoluble components have been studied and the miscibility of components in the monolayer and the phase transition of the monolayer have been explained by applying the phase rule and the theory of regular solution to the monolayer.[8-9] Moreover, the surface tension of aqueous solutions of disodium dodecyl, tetradecyl, and hexadecyl phosphates have been measured. On the basis of the surface tension measurement, it has been found that the adsorption to the solution surface and the formation of micelles of these bi-valent anionic surfactants could be explained satisfactorily by using the activity, instead of the concentration, of these compounds in the solutions,[10] and also that, on the basis of the measurement of the surface tension of a mixed solution of these compounds, the mixed adsorption and the formation of the mixed micelle could be explained by assuming an ideal mixing at the adsorption layer and the micelle phase.[11] The penetration of the soluble component into the insoluble monolayer will be discussed here, with reference to the results of our previous papers.[8-10]

In order to discuss the above subjects quantitatively, the calculation of the penetration amount (the adsorption amount) of the soluble component into the insoluble monolayer is indispensable. The adsorption equation of the soluble component was presented by B. A. Pethica and applied to the system of cholesterol and sodium dodecyl sulfate (SDS).[2]

In this work, the surface pressure of the cholesterol (CH), cholesteryl acetate (CA), and cholesteryl propionate (CP) spread as monolayers on an aqueous solution of disodium hexadecyl phosphate (SHP), was measured by the Wilhelmy method at 25 °C, and the penetration amounts of SHP into the monolayers of cholesterol and its esters were calculated.

Experimental

Materials. The specimen of CH was from the Sigma Chemical Co. and the CP, from The Aldrich Chemical Co. Inc. the CA, from the Nakarai Chemical Co., was recrystallized from acetone. The specimen of SHP was the same as that used in a previous paper, which had been synthesized and dissolved in water, as was reported there.[10]

Surface-pressure Measurement. On the aqueous solution of SHP in a trough, the monolayer of CH, CA, or CP was spreaded by using benzene as the spreading solvent. The curves of the surface pressure against the area per molecule (the F–A curve) was measured by the Whilhelmy plate method by compressing the monolayer with a barrier, that means, by the surface-area-decreasing method (SAD method). Otherwise, F–A curve was obtained by dropping a benzene solution of an insoluble component onto a constant area, that means, by the insoluble-component-addition method (ICA method). The area per molecule, A, is S/nN_A, where S is the area of the aqueous surface between two barriers, n is the mole of the insoluble component spread in this area, and N_A is the Avogadro number. Therefore, A can be decreased either by decreasing S (SAD method) or by increasing n (ICA method). Details of the surface pressure measurement have been reported in a previous paper.[12]

Results

F–A Curves and γ–c_2 Curves of the Monolayers of CH on the Aqueous Solution of SHP. The surface pressure, F, was measured by changing the area per molecule of the insoluble component, A, on the aqueous solution of SHP at various concentrations, c_2. Both the SAD method and the ICA method were applied. The F–A curves of the CH monolayers are shown in Fig. 1, where the surface pressure, F, is defined as usual by:

$$F = \gamma_w - \gamma \qquad (1)$$

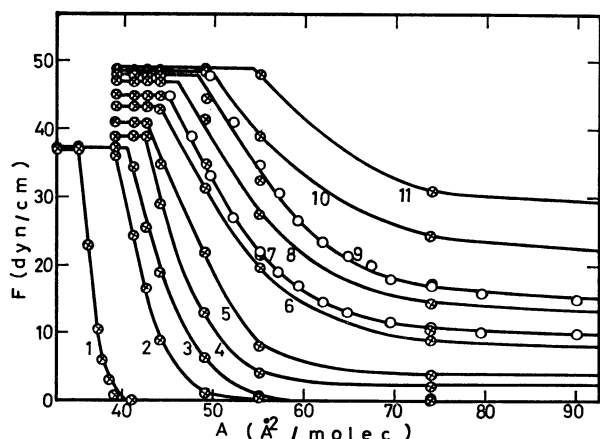where γ is the surface tension of the SHP solution, whose

Fig. 1.　$F-A$ curves of the CH monolayers on the SHP solutions.

$\bigcirc$: SAD method, $\otimes$: ICA method,
c_2: (1) 0 mM, (2) 0.02 mM, (3) 0.05 mM, (4) 0.1 mM, (5) 0.2 mM, (6) 0.4 mM, (7) 0.5 mM, (8) 0.8 mM, (9) 1 mM, (10) 2 mM, (11) 3 mM.

surface is covered with an insoluble monolayer, and γ_w is that of pure water. It is found that the monolayer is more expanded and is shifted to a higher surface pressure on a solution with a higher concentration of SHP, c_2. Even if the practical surface pressure, F', is calculated by subtracting the surface pressure of the SHP solution, the curves of F' against the area per molecule are also more expanded on a solution with a higher value of c_2.[9] This phenomenon is due to the penetration of the soluble component, SHP, into the insoluble monolayer. On the other hand, each curve in Fig. 1 has a kink point. It was found in our previous paper that this point indicates the collapse of the monolayer.[9]
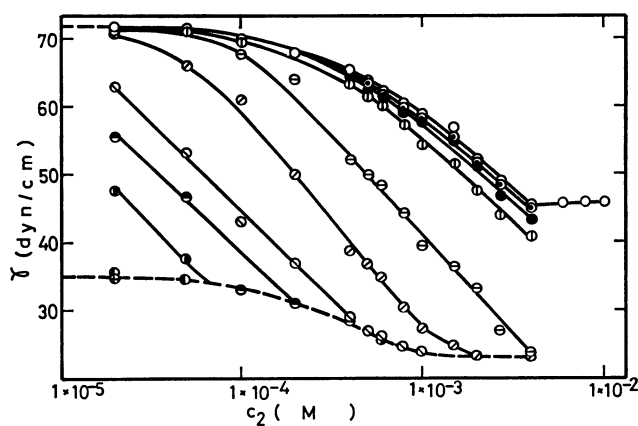

Fig. 2.　The relations of surface tension, γ, and the concentration of SHP, c_2, at various values of A of CH. A (Å^2/molec): $\bigcirc$ no insoluble monolayer, $\odot$ 220, $\bullet$ 110, $\oplus$ 74, $\ominus$ 55, $\oslash$ 49, $\oslash$ 44, $\ominus$ 42.5, $\oslash$ 41, $\oplus$ 39, $\oplus$ 35.

In Figure 2, the surface tension, γ, is plotted against the SHP concentration, c_2. Each line is drawn at a constant area per molecule of the insoluble component (CH), A. The data in Fig. 2 can be transcribed into the data in Fig. 1. The critical micelle concentration of SHP is 6 mM, and the insoluble monolayers were spread

on a SHP solution of less than 6 mM. It was found that, at a constant value of A, the surface tension, γ, decreases with c_2, and that at a constant value of c_2, γ increases with A. The collapse points are shown by the broken line in Fig. 2.

$\gamma-c_2$ _Curves of the Monolayer of CA._　　The $F-A$ curves and the $F'-A$ curves of CA on aqueous solutions of SHP with various values of concentration, c_2 were presented in our previous paper.[9] Both the SAD method and the ICA method were applied; the data based on these two methods were in good agreement. Each $F'-A$ curve had a kink point, which was considered to be the collapse point, and the practical surface pressure of this point (the collapse pressure) was independent of c_2. As well as the monolayer of CH, the monolayer of CA was the more expanded on the SHP solution with a higher concentration.
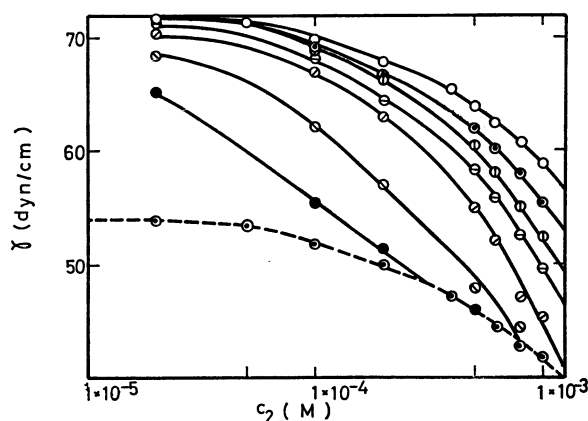

Fig. 3.　The relations of surface tension, γ, and the concentration of SHP, c_2, at various values of A of CA. A (Å^2/molec): $\bigcirc$ no insoluble monolayer, $\odot$ 100, $\oplus$ 80, $\ominus$ 70, $\oslash$ 60, $\oslash$ 50, $\bullet$ 45, $\odot$ 40.

Figure 3 shows the relation between the surface tension, γ, and the SHP concentration, c_2, at various values of the area per molecule of CA, A. The collapse points of the monolayers are shown by the broken line. The general features of the CA monolayers on the SHP solutions are identical with those of the CH monolayers.

$\gamma-c_2$ _Curves of the Monolayers of CP._　　The $F'-A$ curves of the CP monolayer on the aqueous solution of SHP were also presented in our previous paper.[9] Both the SAD method and ICA method were applied here also. It is noticeable that the data based on these two methods were not in agreement. The monolayers of CP on the SHP solutions were easily supercompressed when the SAD method was applied. The value of the surface pressure of the kink point decreased with the time and was constant at $F'=7.5$ dyn/cm. On the other hand, the $F'-A$ curves based on the ICA method showed no change with the time, agreed well with the curves obtained by the SAD method in the region of low F' values, and gave the collapse pressure $F'=7.2$ dyn/cm, which is practically equal to the above-mentioned value of the "equilibrium" collapse pressure obtained by the SAD method.

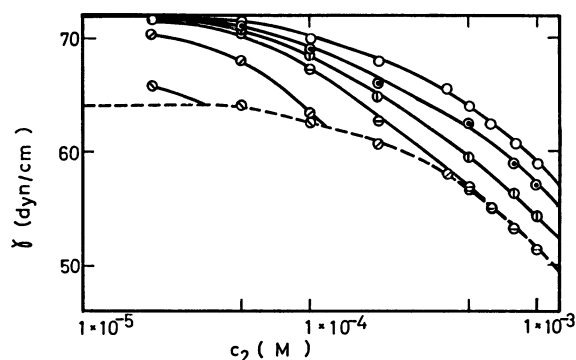In Fig. 4, the relation between the surface tension, γ, and the SHP concentration, c_2 of the equilibrium

Fig. 4. The relations of surface tension, γ, and the concentration of SHP, c_2, at various values of A of CP. A (Å²/molec): ○ no insoluble monolayer, ⊙ 100, ◑ 80, ⊖ 70, ⊘ 60, ◓ 50.

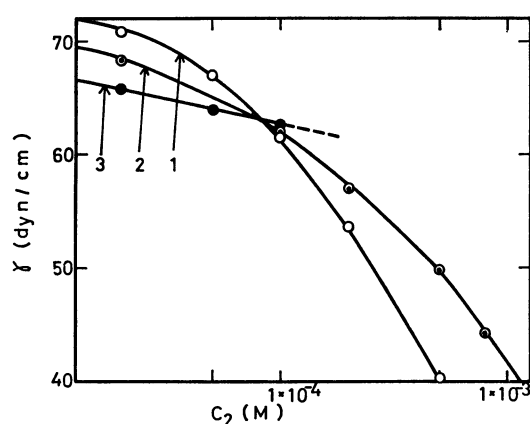Fig. 6. The general features of F-A curves of the penetrated monolayers.

Fig. 5. The relations of surface tension, γ, and the concentration of SHP, c_2, at $A=50$ Å²/molec. Insoluble component: 1. CH, 2. CA, 3. CP.

monolayers of CP are shown. The equilibrium collapse points are shown by the broken line. The general features of the CH, CA, and CP monolayers on the SHP solution are similar. However, there is one noticeable aspect: in comparing at a constant area per molecule of the insoluble components, A, the surface tension is higher in the region of the lower value of c_2 in the order of CH, CA, and CP whereas the surface tension is lower in the region of the higher values of c_2 in the order of CH, CA, and CP. This aspect is shown in Fig. 5, at $A=50$ Å²/molec., as an example. This must affect the penetration isotherms of SHP into the insoluble monolayers of CH, CA, and CP.

Discussion

Mixed Monolayers of an Insoluble Component and a Soluble Component.   Into the insoluble monolayer, the surface-active soluble component, SHP, penetrates and a mixed monolayer of these components is formed. The molecular interaction between the insoluble (CH, CA, or CP in this case) and soluble (SHP in this case) components is important in helping one to decide the general features of the mixed monolayer. Three typical cases, shown in Fig. 6, can be expected.

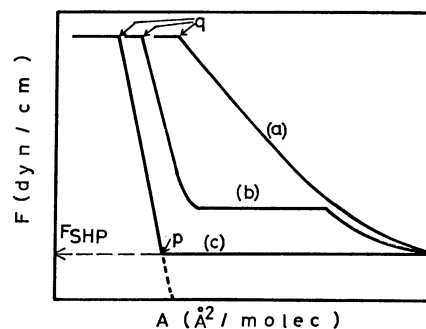a)   If these two components are completely miscible

in the monolayer, the F-A curve of the mixed monolayer takes the shape of Type a.   b)   If these two components are only partially miscible, the F-A curve takes the shape of Type b.   In the plateau region, two monolayer phases of different compositions co-exist under a constant pressure, a constant temperature, and a constant concentration of SHP, c_2.   According to the surface-phase rule, the freedom of the system becomes zero and the surface pressure, F, does not change.[13,14] By the compression of the monolayer, one monolayer phase diminishes, after the complete disappearance of this monolayer phase, the surface pressure again increases.   c)   When the insoluble and soluble components are completely immiscible, the general feature of the F-A curve is Type c.   The surface pressure does not change from F_{SHP} by the compression of the monolayer until the p point.   At this point, the F-A curve is the same as that on pure water (the broken line in in Fig. 6).

On the basis of the above discussion, all of the systems in this paper (CH–SHP, CA–SHP, and CP–SHP) can be identified as of Type a.   Therefore, in the monolayers, the insoluble component and the soluble component are miscible.   The q point is the collapse point of the monolayer.

The Calculation of the Amount of Penetration of SHP into the Insoluble Monolayer.   SHP and the insoluble component (CH, CA, or CP) are designated as Components 2 and 3 (Component 1 is water).   The Gibbs adsorption equation is given as:

$$dF = \Gamma_2 d\mu_2 + \Gamma_3 d\mu_3{}^s$$
$$= \Gamma_2 RT\,d\ln a_2 + \Gamma_3 d\mu_3{}^s \qquad (4)$$

where Γ_2 is the penetration amount (the adsorption amount) of SHP (mol/cm²), a_2 is the activity of SHP in the aqueous solution, Γ_3 is the mole of the insoluble component in the monolayer of 1 cm² and is equal to $1/AN_A$, and $\mu_3{}^s$ is the chemical potential of the insoluble component in the monolayer.   The activity of SHP in the solution, a_2, is defined as:

$$a_2 = \gamma_\pm{}^3 c_2(2c_2+c')^2 \qquad (5)$$

where $\gamma_\pm$ is the mean ionic activity coefficient, the latter can be calculated from the Güntelberg approximation of the Debye-Hückel theory:

$$\log \gamma_\pm = \frac{-B\sqrt{J}}{1+\sqrt{J}} \qquad (6)$$

Here, J is the ionic strength, and B is a constant whose value is 1.022 for SHP at 25 °C.[15] c' is the concentration of the added electrolyte (in this case, $c'=0.01$ M NaOH). With respect to the chemical potential of the insoluble component in the monolayer, $\mu_3{}^s$, we give the following equation:

$$d\mu_3{}^s = N_A\omega_3 dF + RT\,d\ln X_3 \tag{7}$$

Here, N_A is the Avogadro number, ω_3 is the partial molecule area of component 3, which is obtained from the F–A curve of the monolayer on pure water at the corresponding value of the surface pressure, and X_3 is the mole fraction of the insoluble component in the monolayer. X_3 is defined as:

$$X_3 = \frac{\Gamma_3}{\Gamma_2 + \Gamma_3} \tag{8}$$

Because the surface-pressure measurement is performed by changing the concentration of SHP, c_2 (the activity of SHP, a_2), and the area per molecule of the insoluble component, A, under a constant temperature and constant pressure, F and X_3 are functions of $\ln a_2$ and A respectively.

$$dF = \left(\frac{\partial F}{\partial \ln a_2}\right)_A d\ln a_2 + \left(\frac{\partial F}{\partial A}\right)_{a_2} dA \tag{9}$$

$$dX_3 = \left(\frac{\partial X_3}{\partial \ln a_2}\right)_A d\ln a_2 + \left(\frac{\partial X_3}{\partial A}\right)_{a_2} dA \tag{10}$$

Under the constant area per molecule of the insoluble component, A, Eqs. 4, 7, 9, and 10 give the following equation:

$$\Gamma_2 = \frac{(1-\omega_3/A)(\partial F/\partial \ln a_2)_A}{1-(\partial \ln X_2/\partial \ln a_2)_A} \tag{11}$$

Because the value of $(\partial \ln X_2/\partial \ln a_2)_A$ is not directly known from the data (Figs. 2 and 4), we start the calculation of Γ_2 with an approximate equation and revise succesively. For this purpose, the following equation may be used instead of Eq. 11:

$$\Gamma_2 = (1-\omega_3/A)(\partial F/\partial \ln a_2)_A \tag{12}$$

if the following equation is used instead of Eq. 7:

$$d\mu_3{}^s = N_A\omega_3 dF \tag{13}$$

The same equation as Eq. 12 was used by Pethica[2] and other investigators[16] for the systems of cholesterol and SDS and of octadecanol and hexadecyl-trimethyl ammonium bromide. The calculation of Γ_2 from Eq. 11 is performed as follows:

1. Γ_2 is calculated by applying Eq. 12 to, for example, Fig. 3. 2. $\ln X_2$ is calculated by means of Eq. 8. 3. $(\partial \ln X_2/\partial \ln a_2)_A$ is calculated graphically. 4. Now, Γ_2 is recalculated by means of Eq. 11. 5. The successive approximation is repeated until the value of Γ_2 converges to a difined value.

In Fig. 7, the values of Γ_2 calculated from Eqs. 11 and 12 are shown for the $A=220$ Å²/molec of CH as an example. In the region of low concentrations of SHP, c_2, an appreciable difference is recognized between the values calculated from these equations. This is caused by the comparatively large value of $(\partial \ln X_2/\partial \ln a_2)_A$ in this region of c_2. In Fig. 8, the converged values of Γ_2 are shown against the concentration of SHP at various values of the A of CH. The following points are
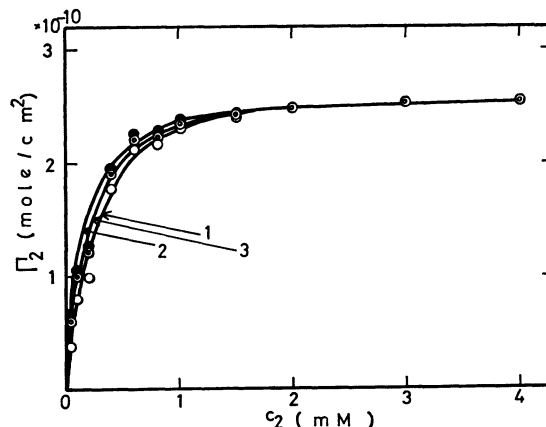

Fig. 7. The relations of the penetration amount, Γ_2, and the concentration of SHP, c_2, for $A=220$ Å²/molec of CH. ◯ 1st approximation by Eq. 12, ● 2nd approximation, ◎ the converged values by Eq. 11.
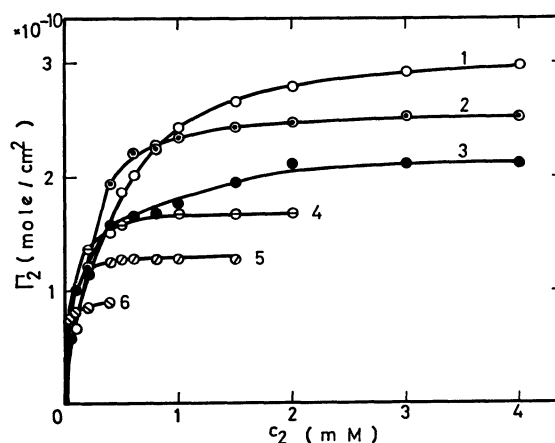

Fig. 8. The relations of the penetration amount, Γ_2, and the concentration of SHP, c_2.
The converged values, A (Å²/molec): 1. no insoluble monolayer, 2. 220, 3. 110, 4. 55, 5. 49, 6. 44. the insoluble component is CH.

found: 1. The relation of Γ_2 and c_2 at each value of A are, in appearance, those of the Langmuir adsorption. 2. The saturated amount of the penetration is larger at a larger value of A. 3. The initial increment of Γ_2 against c_2 is larger at a small value of A.

Because SHP is an electrolyte, we assumed the modified Langmuir adsorption equation by using the activity, a_2, instead of the concentration, c_2:

$$\Gamma_2 = \frac{\Gamma_s K a_2}{1 + K a_2} \tag{14}$$

where Γ_s is the saturated amount of the penetration of SHP and K is the modified Langmuir coefficient. If we, therefor, plot the value of (a_2/Γ_2) against a_2, a straight line is obtained: the values of Γ_s and K are obtained from the slope and the intersection in relation to the ordinate. In Fig. 9, (a_2/Γ_2)–a_2 plots for CH are shown for several values of A. All the plots shown a linear relation: the values of Γ_s and K thus obtained are shown in Fig. 10. The data of CA and CP as well as CH are shown in this figure.
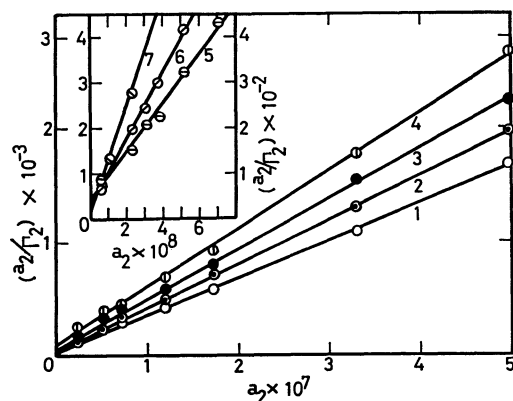
Fig. 9. The Langmuir plots, (a_2/Γ_2) vs. a_2.
The insoluble component is CH. A (Å²/molec): 1. no insoluble monolayer, 2. 220, 3. 110, 4. 74, 5. 55, 6. 49, 7. 44.
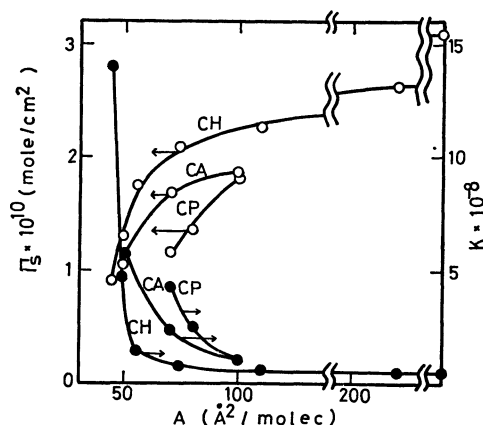

Fig. 10. The saturated penetration amount, Γ_s, or the modified Langmuir coefficient, K, vs. the area per molecule of the insoluble component, A.

The value of the saturated amount of the penetration, Γ_s, is 3.1×10^{-10} mol/cm² when surface is free from the insoluble component, and it decreases with a decrease in the value of A. At a constant value of A, the value of Γ_s is larger in the order of CH, CA, and CP. On the other hand, the value of K is nearly constant at the large value of A $(A > 100$ Å²/molec); it is 0.5—1.0×10^8, which agrees with the value of 0.5×10^8 for

the solution surface free from the insoluble component. When the value of A decreases further, the value of K steeply increases. The value of K is correlated with the strength of the penetration, and the increase in the value of K means a larger strength of the penetration of SHP into the insoluble monolayer and is caused by the greater amount of intermolecule interaction. The value of K for the system of CP–SHP is the largest, and that for the system of CH–SHP is smallest. This is caused by the hydrophobic nature of these compounds; the noticeable difference in Figs. 2, 3, 4, and 5 directly indicate these effect of the K and Γ_s of CH, CA, and CP.

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