A Study of Monolayer Penetration by the Radiotracer Method

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The phenomenon of the penetration of insoluble monolayers of cholesterol, hexadecyl alcohol, etc., by long-chain water-soluble compounds such as sodium salts of long-chain alkyl sulfates was first discovered by Schulman and Hughes.[1] Since then, this phenomenon has been extensively investigated by Schulman and his co-workers, and it has been concluded that stoichiometric complexes between insoluble and soluble species are formed.[1]

Harkins, however, has questioned this idea of complex formation, because the surface tension data observed by Schulman et al. were not those of the true equilibrium.[2] Hutchinson has also shown that the surface tension of aqueous solutions containing octyl alcohol and sodium dodecyl sulfate in various mole ratios does not exhibit the obvious variation which would suggest the stoichiometric complex formation.[3]

Opposing these papers, Matalon[4] and Goddard and Schulman[5] have published experimental evidence for their own views as follows: "By injecting sodium hexadecyl sulfate (SHS) below cholesterol monolayer at constant surface pressure the occupied area of cholesterol molecule increases and its rapid initial increase is from 20 to 27 Å^2. Since this increase approximately corresponds to a cross-sectional area of SHS, a 1 : 1 complex is formed. For hexadecyl alcohol monolayer this rapid initial increase does not correspond to a 1 : 1 complex, but a considerable quantity of SHS is held and stabilized in the surface by hexadecyl alcohol. These complexes are stable up to the collapse points of the mixed monolayers." [5]

Dervichian has considered the monolayer penetration as a partition of soluble species between the surface phase containing the monolayer and the substrate phase. On the basis of this idea he has suggested that certain particular patterns in the mutual arrangement of the molecules appear and that, in the case of the penetration of the hexadecyl alcohol monolayer by SHS, 1 : 1, 1 : 2 and 1 : 3 associations exist.[6]

The thermodynamics of monolayer penetration has been studied by Pethica and Anderson[7] and by Fowkes.[8,9] Especially, Fowkes has asserted that the mixed film resulting from the penetration may be regarded as an ideal mixture rather than as a stoichiometric complex.

The above-mentioned arguments have been based on the data obtained by the measurement of the surface pressure, potential and viscosity. However, data decisive enough to persuade us have not been reported yet on either side of the question. If detergent molecules penetrate into monolayers to form 1 : 1 complexes, the penetrated amount should be of the order of 10^{-10} mol./cm^2, which is enough to be detectable by means of the radiotracer method. Hence, the direct measurements of the amount of some long-chain alkyl sulfates penetrating cholesterol and hexadecyl alcohol monolayers were carried out by the radiotracer method, using ^{35}S-labeled detergents. In addition, octadecyl amine, which is known to form a 1 : 1 salt with these anionic detergents in a bulk solution, was used as a reference monolayer.

A short communication has already come from our laboratory on this subject.[10]

Experimental

Materials.—The ^{35}S-labeled sodium dodecyl sulfate (SDS), sodium tetradecyl sulfate (STS) and sodium hexadecyl sulfate (SHS) used were synthesized from the corresponding fatty alcohols and ^{35}S-labeled sulfuric acid according to the published method.[11]

Pure fatty alcohols were obtained from commercial alcohols by fractional distillation through a

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[1] J. H. Schulman and A. H. Hughes, *Biochem. J.*, **29**, 1243 (1935); J. H. Schulman, *Trans. Faraday Soc.*, 33, 1116 (1937); J. H. Schulman and E. K. Rideal, *Proc. Roy. Soc.*, **B122**, 29, 46 (1937); J. H. Schulman and E. Stenhagen, ibid., **B126**, 356 (1938); J. M. Marsden and J. H. Schulman, *Trans. Faraday Soc.*, **34**, 748 (1938).

[2] W. D. Harkins, "Physical Chemistry of Surface Films," Reinhold Publ. Corp., New York, N. Y. (1952).

[3] E. Hutchinson, *J. Colloid Sci.*, **3**, 413 (1948).

[4] R. Matalon, ibid., **8**, 53 (1953).

[5] E. D. Goddard and J. H. Schulman, ibid., **8**, 309, 329 (1953).

[6] D. G. Dervichian, "Surface Phenomena in Biology and Chemistry," Ed. by J. F. Danielli, K. G. A. Pankhurst and A. C. Riddiford, Pergamon Press, New York, N. Y. (1958), pp. 80—87.

[7] B. A. Pethica, *Trans. Faraday Soc.*, **51**, 1402 (1955); P. J. Anderson and B. A. Pethica, ibid., **52**, 1080 (1956).

[8] F. M. Fowkes, *J. Phys. Chem.*, **65**, 355 (1961).

[9] F. M. Fowkes, ibid., **67**, 1982 (1963).

[10] R. Shimozawa and R. Matuura, This Bulletin, **34**, 457 (1961).

[11] R. Matuura, H. Kimizuka, S. Miyamoto and R. Shimozawa, ibid., **31**, 532 (1958).

rectifying column of 65 plates at a reflux ratio of 15. Fractions which distilled at 130—131°C/8.0 mmHg, 168°C/13.8 mmHg and 181.5—181.7°C/10.0 mmHg for dodecyl, tetradecyl and hexadecyl alcohols respectively were collected. The melting points of these alcohols showed normal values.

The ^{35}S-labeled sulfuric acid was obtained from the Radiochemical Center, England, in a carrier-free state and was isotopically diluted with a small amount of sulfuric acid before use. The specific radioactivity of the ^{35}S-labeled sodium alkyl sulfates thus prepared were ca. 10 mc./mmol.

Octadecyl amine was distilled in the same way as were fatty alcohols, and a fraction which distilled at 198.5—198.8°C/9.2 mmHg was collected. Distilled hexadecyl alcohol and octadecyl amine and the cholesterol of Merck's reagent were all recrystallized from their ethanol solutions and dried in a vacuum. These monolayer substances were spread from benzene solutions.

The water used was redistilled from a quartz still.

Method.—The procedure of measuring the surface excess by the radiotracer method was the same as that in the preceding paper.[12] The surface excess of a detergent at the free surface of its solution, Γ_d (in mol./cm^2), and that at the surface with a monolayer, Γ_m, were calculated according to the following equations:

$$\Gamma_d=\frac{I_d-I}{SA},\quad \Gamma_m=\frac{I_m-I}{SA} \qquad (1)$$

where I_d and I_m are the intensities, in count/min., of the radiation from the detergent solution in the absence and in the presence of a monolayer respectively, and where I is the intensity of the radiation from a sulfuric acid solution, used as a reference, which has the same specific activity and concentration as the detergent solution. S, the specific activity of the detergent in count/min./mol., and A, the surface area of the solution in cm^2, were determined by using a point source, as has been reported previously.[12]

The measurement of the surface pressure was simultaneously carried out by a modified Wilhelmy-type balance.[13]

All experiments were carried out at 35°C.

Results and Discussion

So far various insoluble and soluble substances have been used in the study of the monolayer penetration. The aim of this study was, however, to obtain more exact information on the phenomenon of the monolayer penetration itself. Hence, cholesterol and hexadecyl alcohol as insoluble species and sodium alkyl sulfates as soluble species were used as typical examples.

Three kinds of experimental procedures were applied in order to obtain exact information on the mechanism of the monolayer penetration. Namely, the surface pressure and the surface excess of the detergent as a function of the surface area or of time were measured with (1) the monolayer spread on the detergent solution; (2) the monolayer under which the detergent solution was injected, and (3) the monolayer formed by the spreading of a film substance-detergent mixture on water.

Since the results obtained for three sodium alkyl sulfates are qualitatively similar, the results for SHS only will be described in detail.

The Monolayers Spread on the Surface of the Detergent Solution.—In order to minimize the effect of adventitious ionic contamination, the experiments were carried out on the SHS solution with 10^{-3} mol./l. sodium chloride for cholesterol and hexadecyl alcohol monolayers, and on that with 10^{-3} mol./l. hydrochloric acid for the octadecyl amine monolayer, as was done by Goddard and Schulman.[5] On the other hand, the experiment with the octadecyl amine nonolayer was also carried out on the SHS solution without an added electrolyte.

Prior to the penetration experiment, the measurements were carried out on the free surface of the SHS solution. After sweeping the surface of the SHS solution, the surface excess of SHS at the free surface, Γ_d, and the surface pressure, Π, were measured simultaneously. It was found that both values increase with time and that the former reaches equilibrium within an hour, while the latter still increases very slowly. Two hours after the sweeping, the Γ_d and the change in Π with compression, $\Delta\Pi$, were measured by compressing the free surface of the SHS solution. The

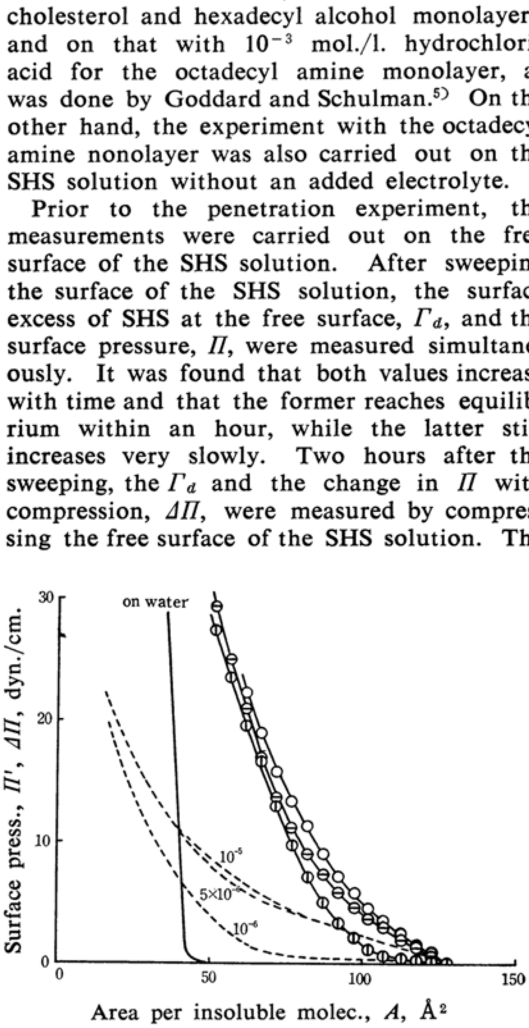

Fig. 1. Π'-A curves for cholesterol monolayers on 10^{-6}, ⦶; 5×10^{-6}, ⊖; and 10^{-5}, ○, mol./l. SHS solutions with 10^{-3} mol./l. NaCl. Broken lines represent the increase in the surface pressure due to the compression of the free surface of each substrate solution.

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[12] A. Matsubara, R. Matuura and H. Kimizuka, ibid., **38**, 369 (1965).

[13] R. Matuura and I. Hayashi, *Memoirs Fac. Sci., Kyushu Univ. C*, **1** (1948).

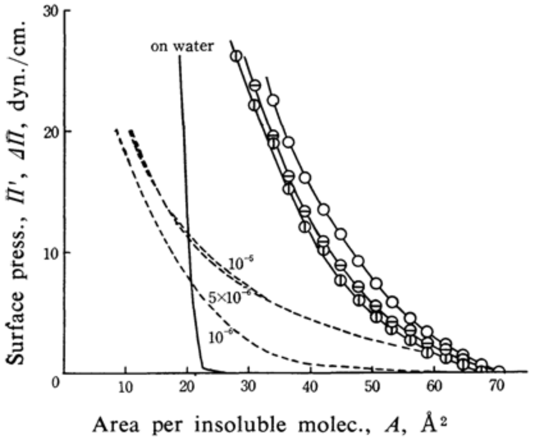


Fig. 2. Π'-A curves for hexadecyl alcohol monolayers on 10^{-6}, ⦶; 5×10^{-6}, ⊖; and 10^{-5}, ○, mol./l. SHS solutions with 10^{-3} mol./l. NaCl. Broken lines represent the increase in the surface pressure due to the compression of the free surface of each substrate solution.

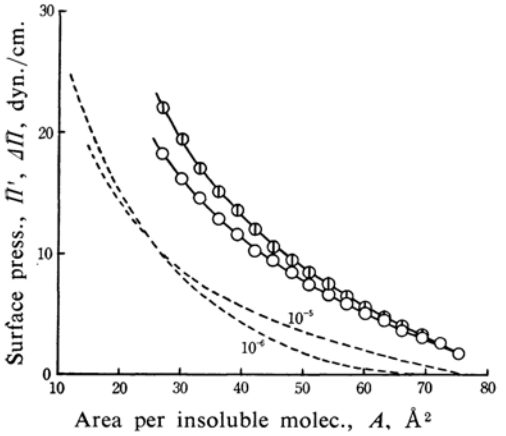


Fig. 3. Π'-A curves for octadecyl amine monolayers on 10^{-6}, ⦶; and 10^{-5}, ○, mol./l. SHS solutions with 10^{-3} mol./l. HCl. Broken lines represent the increase in the surface pressure due to the compression of the free surface of each substrate solution.

compression was carried out at the rate of 1 cm. of the trough scale a minute (trough: $15\times30\,\text{cm}^2$) and at three-minute intervals.

At the end of the measurements on the free surface of the SHS solution, the surface was again swept and the monolayer was spread as quickly as possible. Two hours after the spreading, the values of Γ_m and the surface pressures referred to each substrate solution, Π', were measured as a function of the occupied area of the insoluble molecule by compressing the monolayer at the same rate and interval as in the measurement with the free surface.

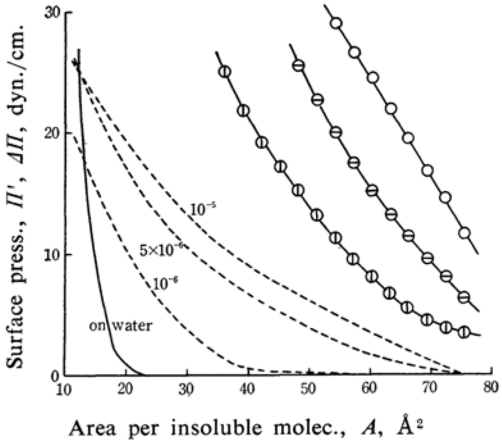


Fig. 4. Π'-A curves for octadecyl amine monolayers on 10^{-6}, ⦶; 5×10^{-6}, ⊖; and 10^{-5}, ○, mol./l. SHS solutions without added electrolyte. Broken lines represent the increase in the surface pressure due to the compression of the free surface of each substrate solution.

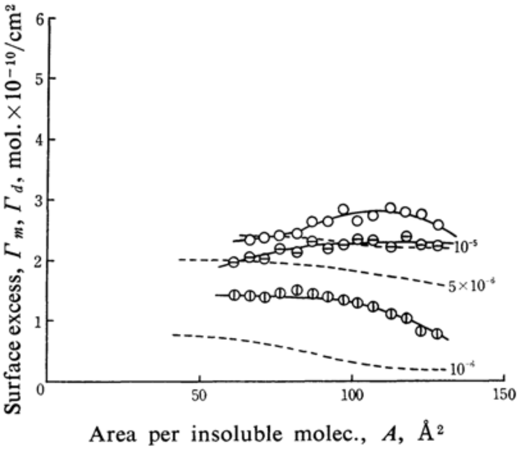


Fig. 5. Γ_m-A curves for cholesterol monolayers on 10^{-6}, ⦶; 5×10^{-6}, ⊖; and 10^{-5}, ○, mol./l. SHS solutions with 10^{-3} mol./l. NaCl. Broken lines represent the change in the surface excess due to the compression of the free surface of each substrate solution.

The results are shown in Figs. 1—4 (for $\Delta\Pi$ and Π') and in Figs. 5—8 (for Γ_d and Γ_m).

It should be noticed in these figures that, with the compression of the free surface of the SHS solution, the surface pressure increased with a considerable stability, as is shown by the broken lines in Figs. 1—4, while there was little change in the surface excess, as Figs. 5—8 show. It may also be seen in these figures that cholesterol and hexadecyl alcohol monolayers spread on the SHS solutions expanded two or three times in comparison with those on water, while the surface excess of SHS did

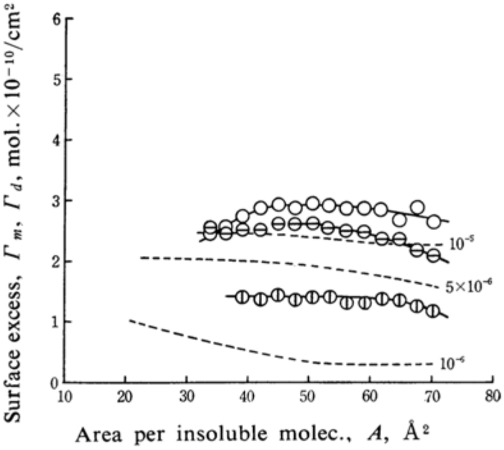


Fig. 6. Γ_m-A curves for hexadecyl alcohol monolayers on 10^{-6}, ⦶; 5×10^{-6}, ⊖; and 10^{-5}, ○, mol./l. SHS solutions with 10^{-3} mol./l. NaCl. Broken lines represent the change in the surface excess due to the compression of the free surface of each substrate solution.

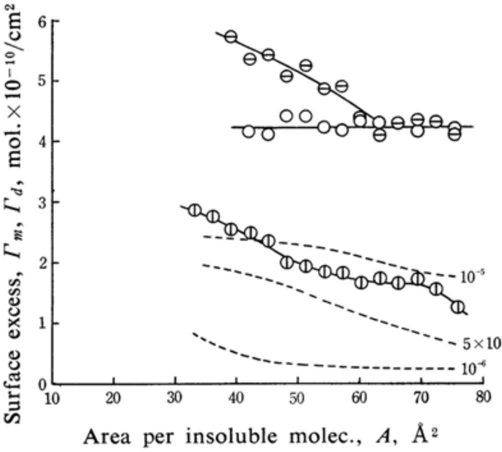


Fig. 8. Γ_m-A curves for octadecyl amine monolayers on 10^{-6}, ⦶; 5×10^{-6}, ⊖; and 10^{-5}, ○, mol./l. SHS solutions without added electrolyte. Broken lines represent the change in the surface excess due to the compression of the free surface of each substrate solution.

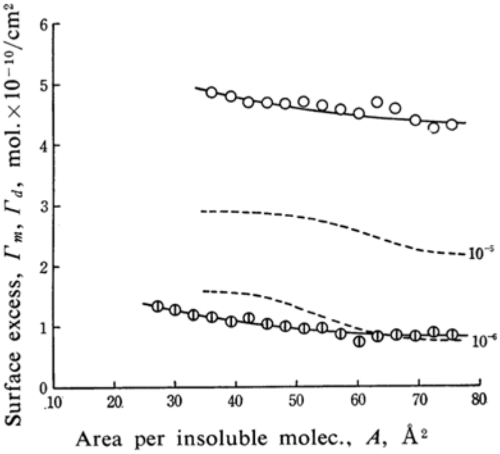
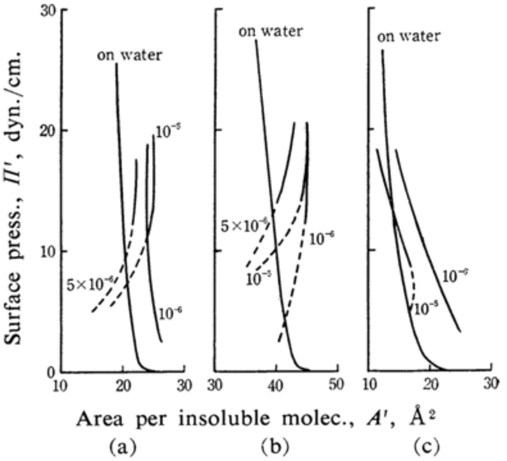


Fig. 7. Γ_m-A curves for octadecyl amine monolayers on 10^{-6}, ⦶; and 10^{-5}, ○, mol./l. SHS solutions with 10^{-3} mol./l. HCl. Broken lines represent the change in the surface excess due to the compression of the free surface of each substrate solution.



(a) (b) (c)

Fig. 9. (a) and (b) represent Π'-A' curves for hexadecyl alcohol and cholesterol monolayers on SHS solutions with 10^{-3} mol./l. NaCl, respectively and (c) represents that for octadecyl amine monolayer on SHS solutions with 10^{-3} mol./l. HCl.

not increase so much as to explain this large expansion and was insufficient to form the 1 : 1 complex, as is shown by solid lines in Figs. 1—8. Especially at higher concentrations of SHS, the Γ_m's were almost the same as the corresponding Γ_d's, indicating that no extra detergent ion was taken up by the insoluble monolayer.

Since in the surface the adsorbed detergent ions are present in addition to the cholesterol or hexadecyl alcohol molecules, it is quite natural that these monolayers expand remarkably. In addition, it should be noted that the abnormally large expansion of the Π'-A curves, as seen in Figs. 1—4 and in previous papers,[1,5)] involves the effect of the compression of the free surface. In fact, subtracting the area of the free surface from that of the penetrated film at the same surface pressure, we obtain the surface pressure - area curve (Π'-A' curve), as is shown in Figs. 9(a) and (b). These curves indicate only small expansions of the limiting areas of monolayers. The presence of this free surface effect is characterized by the features of the Π'-A curves, indicating an increase in the surface pressure from just the area at which the spreading solution has been dropped, as Figs. 1—4 show. This feature is observed also in the Π-A curves reported by Schulman et al.[1,5] It has been stated[5] that the complex produced by the penetration of the detergent is stable up to the collapse point. This statement is inconsistent with the present findings that the apparent increase in the surface area is mainly due to the compression of the free surface and that the Γ_m is almost constant with the compression of the monolayer and never reaches the amount corresponding to the 1 : 1 complex. Further, the Π'-A curves of the monolayers on the SHS solution are strongly time-dependent. From these reasons it should be concluded that the Π'-A curves obtained by compressing the insoluble monolayer on the SHS solution can not be used for the thermodynamic consideration.

In the experiments with the octadecyl amine monolayer spread on the SHS solution without hydrochloric acid, the Π'-A curves showed a much more remarkable expansion than those of cholesterol and hexadecyl alcohol monolayers, and the surface excess of SHS also increased pronouncedly in comparison with that in the case of the free surface. The differences between Γ_m and Γ_d nearly corresponded to the 1 : 1 salt formation at the 5×10^{-6} and 10^{-5} mol./l. concentrations of SHS and over a larger area. However, these differences are almost constant, independent of the compression of the monolayer.

In the experiments with the octadecyl amine monolayer spread on the SHS solution containing 10^{-3} mol./l. hydrochloric acid, the Π'-A curves are quite similar to those of cholesterol and hexadecyl alcohol monolayers in spite of the fact that the surface excess of SHS corresponds to the 1 : 1 salt formation at the SHS concentration of 10^{-5} mol./l. and over a larger area. In Fig. 10, Π'-A' curves for the octadecyl amine monolayers on SHS solutions, in the absence of hydrochloric acid, are given. As may be seen in this figure, the curves still indicate a remarkable expansion as compared with the monolayer on water. This seems to be a characteristic feature, one which provides us with evidence for the complex formation. However, Π'-A' curves obtained in the presence of 10^{-3} mol./l. hydrochloric acid show only a small expansion, as is shown in Fig. 9(c). These findings suggest that the expansion of the Π'-A' or Π'-A curve cannot be an indication of the complex formation.

The Injection of Detergents below Monolayers.—Insoluble monolayers were spread on the surface of water and compressed up to a surface pressure of 15 dyn./cm. Then the solution of detergents was injected below the monolayer by means of a wide-mouth pipette and the substrate solution was rendered uniform by gentle sucking and blowing for five minutes. The concentration of the injecting solution was twenty-five times as high as the equilibrium concentration of the substrate solution, and its volume was 10 cc. The Γ_m's at a constant area were measured as a function of the time. The results are shown in Figs. 11—13 for combinations of three kinds of monolayers and three detergents. It may be seen from these figures that, after the injection of the detergents, the surface excesses of detergents increase with the time. In the case of cholesterol and hexadecyl alcohol monolayers, their equilibrium values are approximately the same as

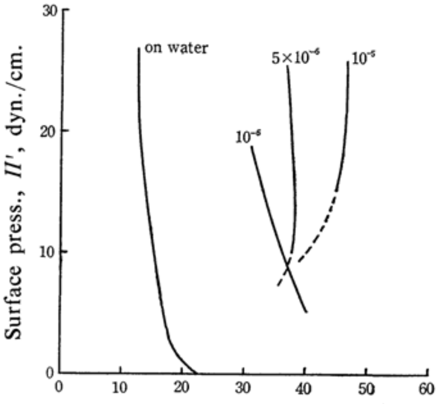


Fig. 10. Π'-A' curve for octadecyl amine monolayer on SHS solutions without added electrolyte.

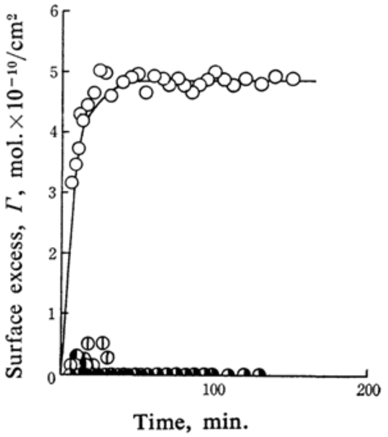


Fig. 11. Injection of SDS below the free surface, ⦶; and cholesterol, ◑; hexadecyl alcohol, ◐; and octadecyl amine, ○, monolayers. The equilibrium concentration of the substrate solution is 10^{-5} mol./l.

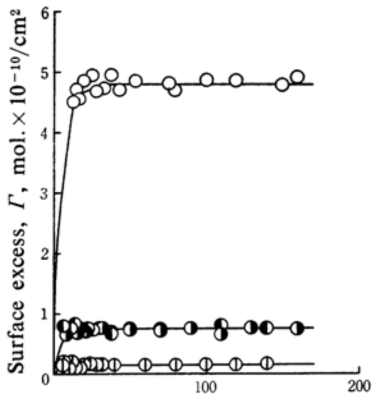


Fig. 12. Injection of STS below the free surface, ⦶; and cholesterol, ◑; hexadecyl alcohol, ◐; and octadecyl amine, ○, monolayers. The equilibrium concentration of the substrate solution is 10^{-5} mol./l.

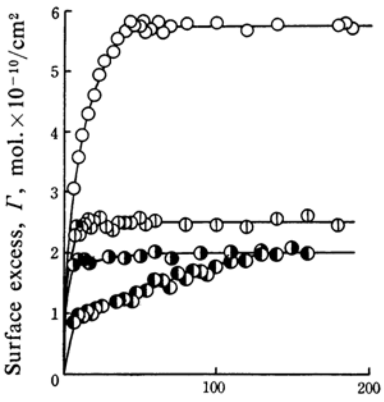


Fig. 13. Injection of SHS below the free surface, ⦶; and cholesterol, ◑; hexadecyl alcohol, ◐; and octadecyl amine, ○, monolayers. The equilibrium concentration of the substrate solution is 10^{-5} mol./l.

that at the surface without a monolayer. On the other hand, in the case of the octadecyl amine monolayer the surface excess increases remarkably beyond that of the free surface. This is incontrovertible evidence that cholesterol and hexadecyl alcohol monolayers do not interact with SHS in such a way as to form the 1 : 1 complex, as has been observed with the octadecyl amine monolayer. It has been suggested[4,5] that, in the experiment with the injection of SHS below the cholesterol monolayer at a constant surface pressure, the rapid initial change in the surface area is due to the 1 : 1 complex formation and that the subsequent slow increase is due to the "solution" of SHS in excess of a 1 : 1 ratio into the monolayer. This interpretation is clearly a misunderstanding. Our suggestion is that the rapid initial change is due to the formation of the adsorbed film of detergents at the surface in the presence of the monolayer, and that the subsequent slow change is to be attributed to the so-called surface ageing which is frequently observed in the surface tension measurement of detergent solutions. Incidentally, the surface potential vs. time curves obtained by Goddard and Schulman[5] are similar to our surface excess vs. time curves; i. e., both curves show a rapid initial change, followed by no slow change.

The Mixed Monolayers of Insoluble and Soluble Species Spread on the Surface of Water.—A mixture of insoluble substances and soluble detergents in the ratio of 1 : 1 was spread on the surface of water by using a mixed solvent composed of benzene, isopropanol and water (25 : 23 : 2 in volume). Thirty minutes after the spreading of the mixture, the surface film was compressed at the same rate and interval as those used previously and the surface concentration, Γ, of the detergent was measured as a function of the occupied area of the insoluble molecule. In these experiments, even if all the molecules of the detergents have dissolved into the substrate, the radiation from the bulk of the substrate is negligibly small, because only a minute amount of detergent has been spread.

The Π-A and Γ-A curves for the mixed monolayers of insoluble species and SHS molecules in the ratio of 1 : 1 are shown in Figs. 14—16. In the case of cholesterol- and hexadecyl alcohol - SHS mixed films, the surface concentrations of SHS are very low, approximately the same as that obtained by spreading SHS only; in addition, there is no appreciable increase in the surface count with the compression of the mixed films. On the contrary, the surface concentration of SHS in the octadecyl amine - SHS mixed film is much higher

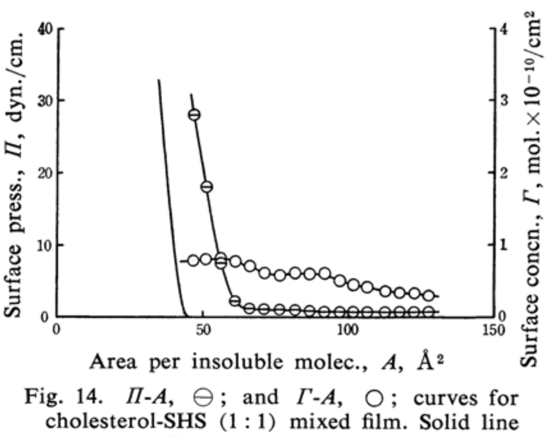


Fig. 14. Π-A, ⊖; and Γ-A, ○; curves for cholesterol-SHS (1 : 1) mixed film. Solid line represents Π-A curve for cholesterol monolayer.

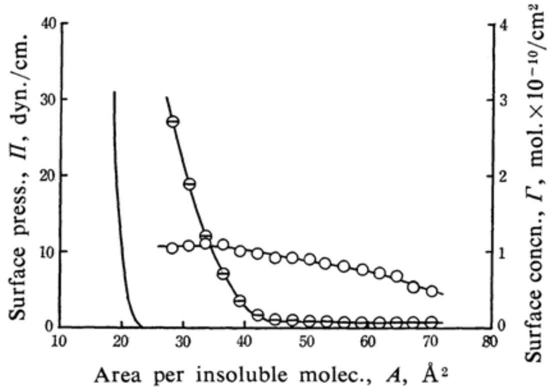


Fig. 15. Π-A, ⊖; and Γ-A, ○; curves for hexadecyl alcohol - SHS (1 : 1) mixed film. Solid line represents Π-A curve for hexadecyl alcohol monolayer.

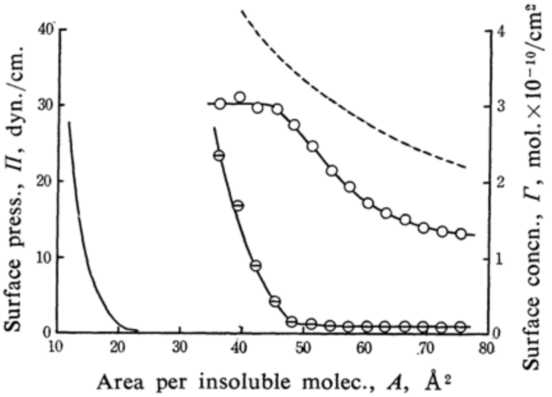


Fig. 16. Π-A, ⦶; and Γ-A, ○; curves for octadecyl amine - SHS (1 : 1) mixed film. Broken line represents an ideal Γ-A curve for 1 : 1 mixed film. Solid line represents Π-A curve for octadecyl amine monolayer.

than those in the case of cholesterol and hexadecyl alcohol and increases in accordance with the increase in the surface concentrations with the compression of the mixed film at a low surface pressure. The mole ratio of SHS to octadecyl amine at the surface is ca. 0.8 at the limiting area. In conformity to this fact, the Π-A curve for the octadecyl amine - SHS mixed film shows a remarkable expansion. It should, however, be pointed out that the increase in the limiting area in the cholesterol- and hexadecyl alcohol - SHS mixed films are ca. 20 Å^2 per insoluble molecule, although the surface concentrations of SHS in these films are much lower than that corresponding to the 1 : 1 complex. This situation is similar to that which holds for the monolayers spread on the SHS solution.

It has been reported by Goddard and Schulman[5] that the result obtained by injecting SHS below the spread cholesterol-SHS mixed film also shows a rapid initial change. This has been considered to be an unexpected result, one inconsistent with the concept they themselves had proposed, that the rapid initial change was due to the 1 : 1 complex formation. This, however, seems quite natural in terms of our interpretation that it is due to the formation of the adsorbed film of SHS. Namely, most of SHS molecules in the spread mixed film have dissolved into the substrate, and only a very low surface concentration of SHS molecules in equilibrium with the bulk concentration remains at the surface. Therefore, if SHS is again injected below such a film, the surface excess of SHS may increase as a result of the increase in the bulk concentration. Hence, a rapid initial change occurs again to form the adsorbed film of SHS.

It may be seen in Fig. 16 that the surface concentration of SHS in the spread octadecyl amine - SHS mixed film does not increase with the compression of the film over areas smaller than the limiting area of the mixed fiilm. As has been mentioned already, in the case of the octadecyl amine monolayer spread on the SHS solution, the surface excess of SHS also shows no increase with the compression of the monolayer. This seems to be due to the collapse of the octadecyl amine - SHS film at a relatively low surface pressure.

When cholesterol- and hexadecyl alcohol - STS mixed films are spread, molecules of STS dissolve almost completely into the substrate. On the other hand, for the octadecyl amine - STS mixed film, molecules of STS corresponding to the ratio of 1 : 1 remain at the surface, as Fig. 17 shows.

All the results obtained in the present experiments clearly indicate that the mode of interaction of sodium alkyl sulfates with the

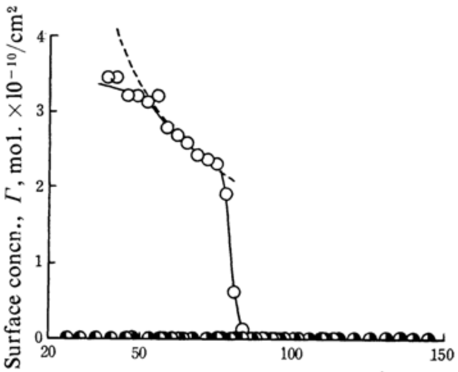


Fig. 17. Γ-A curves for cholesterol -, ◑; hexadecyl alcohol -, ◐; and octadecyl amine - STS (1 : 1), ○, mixed films. Broken line represents an ideal Γ-A curve for octadecyl amine - STS (1 : 1) mixed film.

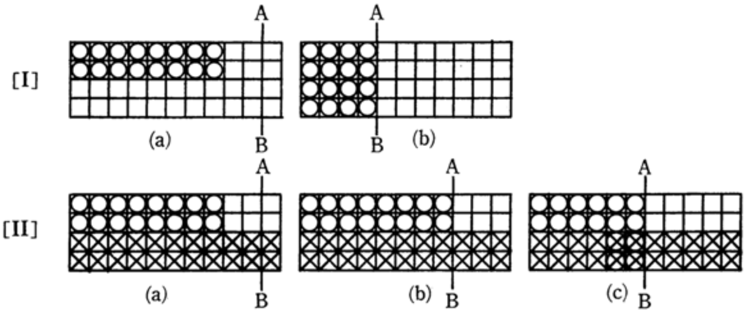


Fig. 18. Compression processes for monolayers [I] on water and [II] on detergent solution, respectively.
(b) represents the closely packed state.
AB, barrier ; ○, insoluble molecule ; ×, detergent molecule

monolayer of cholesterol or hexadecyl alcohol is quite different from that with the monolayer of octadecyl amine. In the latter case the evidence is ample for the formation of the 1 : 1 salt, but we can not obtain any direct evidence for the 1 : 1 complex formation in the former case. Although the true nature of the interaction between sodium alkyl sulfates and cholesterol or hexadecyl alcohol monolayer is as yet obscure, we may suppose that a very weak interaction, such as the van der Waals force, may play a role between two species. But in any way, we can completely deny the well-defined 1 : 1 complex formation.

As Figs. 1—8 show, the surface pressure of the SHS solution, both with and without an insoluble monolayer, increases with compression, while the surface excess does not change appreciably. This phenomenon has not yet been explained completely, but it may be supposed that such a mechanism as, for example, the rearrangement of the adsorbed detergent molecules may influence the kinetic surface pressure, while it has nothing to do with the surface excess. (The disagreement between the surface pressure and the surface potential as regards the time effect had already been noticed by Addison.[14])

On the basis of the experimental results obtained with a cholesterol or hexadecyl alcohol monolayer on detergent solutions in the present study, we may show the change occurring in the monolayer on compression schematically as in Fig. 18. In this figure the II-b state corresponds to the limiting area of the resulting mixed film. This limiting area is larger than that, the I-b state, for the monolayer on water. Therefore, even if the surface excess of SHS at the surface with a monolayer is the same as that at the free surface, the resulting mixed film must expand. This expansion of the monolayer by SHS molecules adsorbed at the surface will be discussed quantitatively.

The Π-A curves of the monolayers on the detergent solutions previously obtained by many workers involve the effect resulting from the compression of the free surface. The reason why the surface pressure increases, while the surface excess of detergent does not, with the compression of the free surface, has not yet been made clear. It is important here that the increase in the surface pressure is not due to the increase in the surface density of SHS. In order to avoid this effect, Harkins et al. have measured the surface pressure by injecting SHS below the hexadecyl alcohol monolayer at each area.[2] These Π-A curves are very different from those obtained by Schulman et al.[1] Therefore, by assuming that the expansions of Π-A curves are due to the presence of SHS molecules adsorbed at the surface, the surface excess of SHS can be estimated from the increase in the limiting area and can be compared with that obtained directly by the radiotracer method.

According to Harkins et al., the limiting areas obtained by extrapolating the linear portions of each curve to the surface pressure of each substrate solution are larger than that for the monolayer on water by ca. 13.5, 24.5 and 25 Å^2 per hexadecyl alcohol molecule for the SHS concentrations of 3×10^{-6}, 10^{-5} and 3×10^{-5} mol./l. respectively. These values represent the difference in the area between the I-b and II-b states in Fig. 18 in Å^2 per hexadecyl alcohol molecule.

In the hexadecyl alcohol monolayer, which is in the closely packed state, there are 5×10^{14} molecules per cm^2. Therefore, the increase in the total surface area of the mixed film per unit area of hexadecyl alcohol molecules is

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[14] C. C. Addison and D. Litherland, *Nature*, **171**, 393 (1953).

$24.5\times5\times10^{-2}$ cm^2 for the SHS concentration of 10^{-5} mol./l. This increase is regarded as being due to the SHS molecules adsorbed at the surface. If it is assumed that the molecular area of SHS is 40 Å^2,[8] this increase in the surface area corresponds to $(24.5\times5\times10^{14})/(40\times6\times10^{23})$ mol. of SHS. Since this quantity of SHS exists within the surface area of $(1+24.5\times5\times10^{-2})$ cm^2, Γ_m is 2.3×10^{-10} mol./cm^2. This value is in excellent agreement with that obtained by our direct measurements, as is shown in Table I. The estimated results for other concentrations of SHS are also shown in Table I.

TABLE I. THE SURFACE EXCESS OF SHS CALCULATED FROM THE DATA OF HARKINS ET AL.

Concn. of SHS mol./l.	ΔA Å^2	Γ_m (Calcd.) mol. $\times10^{-10}$/cm^2	Γ_m (Obs.) mol. $\times10^{-10}$/cm^2
3×10^{-6}	12.5	1.6	1.0
10^{-5}	24.5	2.3	2.0
3×10^{-5}	25	2.3	2.0

These results show that the expansion of the monolayer can be interpreted as being due to SHS molecules adsorbed at the surface, without assuming the 1 : 1 complex formation. Accordingly, it may be expected that the monolayer will expand as the surface concentration of detergent becomes higher because then the surface concentration of species, including both soluble and insoluble species, becomes higher.

By injecting the identical detergent below various monolayers, the increase in the surface pressure has been measured and has been considered as a measure of the interaction of insoluble with soluble species.[1] However, even if the same adsorption of SHS takes place at the surface with different monolayers, the increases in the surface pressure are not necessarily the same. For the monolayers in the closely-packed state especially, the stability of the monolayer and the change in the chemical potential of the monolayer substance due to the adsorption of SHS, etc., which are also responsible for the increase in the surface pressure, should be taken into consideration.

It can be concluded from the foregoing discussion that the so-called reactivity for penetration is nothing but the surface activity of the detergent. In other words, although Schulman et al. have considered the complex formation to be the principal factor in the phenomenon of the monolayer penetration, we consider it to be the surface activity of soluble species. This concept is consistent with these previous experimental data:

(1) The soluble species which are known to cause the monolayer penetration are all surface active substances.

(2) Therefore, the order of the penetration reactivity of detergents for the identical monolayer should be that of the surface activity of soluble species.

a) It has been shown that, for the detergents with a C_{12}-alkyl chain, the order of the penetration reactivity of polar heads is as follows;[15)]

$$-SO_4{}^{2-} > -SO_3{}^{2-} > -NH_3{}^+ > -COO^- > -N(CH_3)_3{}^+$$

By regarding the CMC of detergents as a measure of the surface activity, the order of the penetration reactivity is the same as that for the surface activity of soluble species.

b) For long-chain alkyl sulfates, the longer the alkyl chain, the stronger the penetration reactivity of the detergents.[1] The surface activities of detergents are, of course, in the same order.

c) It has been shown that the penetration reactivity of detergents increases with the addition of salt.[7] This should be attributed to the well-known fact that the addition of salt increases the surface adsorption of detergents.[16]

In the present paper we have pointed out the misinterpretation of the monolayer penetration and have verified that the difference between the properties of the monolayers on the detergent solution and those on water is to be attributed merely to the presence of the detergent molecules adsorbed at the surface.

Finally, it is important to note here that the change in the surface pressure alone in the course of the penetration experiment provides no exact information as to whether the complex is formed or not.

Summary

The surface pressure - area isotherms of monolayers of cholesterol, hexadecyl alcohol and octadecyl amine on sodium alkyl sulfate solutions and the surface excess of alkyl sulfate have been measured by the film balance and the radiotracer methods respectively. These measurements were carried out with (1) the monolayers spread on the alkyl sulfate solutions, (2) those below which the detergent solutions were injected, and (3) those formed by the spreading of the insoluble species-detergent mixtures on water.

All the surface pressure - area curves showed

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[15] B. A. Pethica and J. H. Schulman, *Biochem. J.*, **53**, 177 (1953).

[16] R. Matuura, H. Kimizuka and A. Matsubara, This Bulletin, **34**, 1512 (1961).

remarkable expansions; the results were essentially the same as those observed by Schulman et al. and by others.

On the other hand, the surface excess of alkyl sulfates showed no appreciable increase in the case of cholesterol and hexadecyl alcohol, and it was too low to form a stoichiometric complex between the insoluble species and the alkyl sulfate; only in the presence of the octadecyl amine did it increase to an amount sufficient to form the 1 : 1 stoichiometric salt.

Thus it has been concluded that the expansion of the surface pressure - area curve can not be an indication of the stoichiometric complex formation. It has been pointed out that the surface pressure of the detergent solution increases with the compression, even in the absence of a monolayer; this is the main cause for the expansion of the surface pressure - area curve of the penetrated monolayer. The phenomenon of monolayer penetration has been explained in terms of the formation of the adsorbed layer of alkyl sulfate, and the reactivity for penetration has been related to the surface activity of detergents.

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