Study on Skin Roughness Caused by Surfactants: II. Correlation Between Protein Denaturation and Skin Roughness

G. IMOKAWA, K. SUMURA, and M. KATSUMI, Industrial Research Laboratories,
Kao Soap Co., Ltd., Minatoyakushubata, Wakayama, Japan

ABSTRACT

An attempt to determine protein denaturing potency of typical surfactants has been made by measuring specific rotation of bovine serum albumin. The potencies obtained were examined in relation to the intensities of skin roughness in vivo caused by the surfactants, and a noticeable correlation was found between them. This fact also suggested that the cause of skin roughness may be attributed to a certain extent to adsorption of surfactants. In addition, this technique is very useful in predicting the skin roughening potency of a surfactant without using human skin.

INTRODUCTION

Dermatological problems due to detergents have increased. In particular, a so-called hand roughness which results from a repeated exposure to a detergent solution during dishwashing recently has become a serious problem.

Many investigators, such as Blench, Kirk, Blank, and Vann Scott (1-4) already have reported on the skin roughness, clinically characterized by gross visible changes such as abnormal scaling of the horny layer, that may be caused by the following actions of surfactants: a) removal of lipids from skin surface (1,2); b) washing away of substances such as free amino acids having water binding capacity in the horny layer (3); c) denaturation of epidermal keratin following adsorption of surfactants (4,5); d) inhibition of the enzyme activity in upper layer of skin (6); or e) a cumulative primary irritation (7). However, it still is not certain which action produces the skin roughness, because experiments have never been carried out quantitatively.

Among the 5 actions described above, the third action is particularly interesting in connection with the fact that protein is easily and strongly adsorbed by surfactants and then denatured. Thus it is expected that the action may play an important role in roughening the skin. Therefore, the interaction between surfactants and protein was studied in order to obtain a better understanding of the protein denaturation due to surfactants.

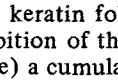
Although the measurement of sulfhydryl group liberated from isolated callus by the action of surfactants is useful in obtaining information on denaturation, the liberation seems not to be essential to the skin roughness. In unpublished data obtained in our laboratory, no measurable amount of the sulfhydryl group was liberated, at least under the mild conditions, such as the skin roughness caused by surfactants, and the extent of sulfhydryl group liberated by harder treatment does not correspond with the intensity of skin roughness.

Thus, the interaction between bovin serum albumin (BSA) and surfactants was chosen as a more sensitive and convenient model for evaluating the protein denaturation. It is well known that a globular protein, such as BSA, easily undergoes denaturation in the presence of excess surfactants, and the specific rotation of the protein increases in levorotation (8). The relationship between specific rotation and protein denaturation has been studied by Doty (9,10). He pointed out that the changes in specific rotation are due mainly to unfolding of the α helix having the nature of dextrorotation, and that the changes occur in proportion to the magnitude of denaturation. By the changes in specific rotation of BSA in the presence of surfactants, therefore, it should be possible to measure the protein denaturing potency of surfactants.

The objective of this paper was to determine the protein denaturing potency of some surfactants by means of the specific rotation, and to discuss the relationship between the protein denaturation and skin roughness.

EXPERIMENTAL PROCEDURES

Materials

The BSA used was obtained through Armour Company (Chicago, IL). Alkyl sulfate (AS) ($C_nH_{2n+1}OSO_3Na$ [C_nAS]), alkyl polyoxyethylene sulfate (ES) ($C_nH_{2n+1}O(CH_2CH_2O)_pSO_3Na$ [C_n-pES]), alkyl benzene sulfonate (LAS) (C_nH_{2n+1}  SO_3Na[C_nLAS]), alfa-olefin sulfonate (AOS) ($C_{n-3}H_{2n-5}CH_2$-CH=CHSO_3Na and $C_{n-3}H_{2n-5}$CH(OH)-CH_2-CH_2SO_3Na [C_nAOS]), paraffin sulfonate (SAS) ($C_nH_{2n+1}SO_3Na$ [C_nSAS]), sodium carboxylate ($C_{n-1}H_{2n-1}COONa$; C_nSoap), alkyl betain (B-n) (C_nH_{2n+1}-$N(CH_3)_2CH_2COO$ [B-n]), alkyl sulfo betain (SB-n) C_nH_{2n+1}-$N(CH_3)_2(CH_2)_3$-SO_3 [SB-n]), and alkyl polyoxyethylene (EO) ($C_nH_{2n+1}O(CH_2CH_2O)_pH$ [C_n-pEO]) are identical with those reported previously (11). Alkyl trimethyl ammonium chloride (TAC) ($C_nH_{2n+1}N(CH_3)_3Cl$ [C_nTAC] was recrystallized 3 times from aceton and alkyl distributions were determined by gas chromatography. The analytical data obtained are shown in Table I.

Measurement of Optical Rotation

BSA solution and various surfactant solutions were prepared by using ion exchanged water and mixed to 1.0g/100ml and 0.1-1.5g/100ml concentrations, respectively. To insure complete interaction, the mixed solutions were allowed to stand overnight at 30 C. Then optical rotations of the solutions were measured with Model DIP-SL automatic polarimeter at 589 nm and 23 C.

Table I

Analytical Data of Alkyl Trimethyl Ammonium Chloride

	Elemental analysis					Distribution of alkyl chain length (%)					
	C (%)	H (%)	N (%)	Cl (%)	Water (%)	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	Purities[a] (%)
C_{12}TAC	66.9	12.4	5.0	14.1	0.64	--	98.93	0.06	0.80	--	94.77
C_{14}TAC	69.3	12.9	4.5	11.5	1.46	0.20	4.20	93.40	1.90	0.30	95.13
C_{16}TAC	69.4	12.7	3.9	12.0	2.82	--	--	1.20	97.12	0.66	91.63

[a]Determined from the data on elemental analysis.

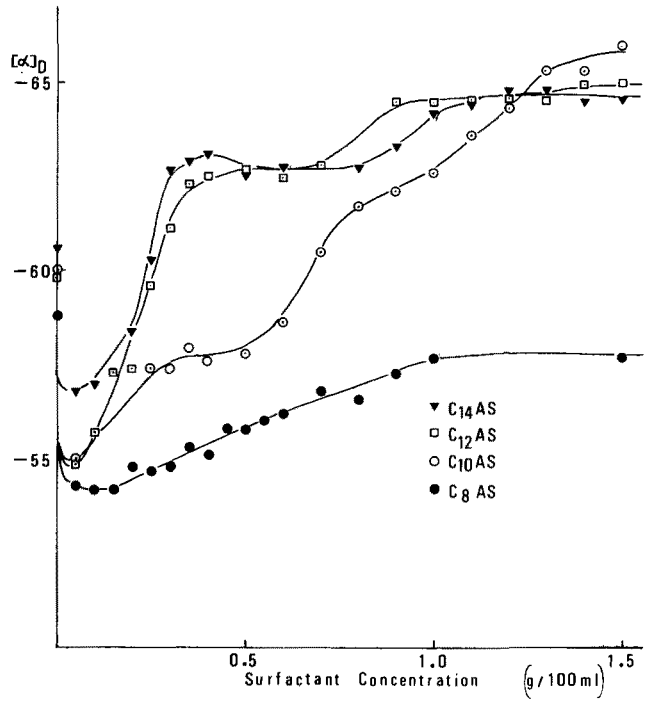


FIG. 1. The specific rotation of bovine serum albumin (BSA) as a function of surfactant concentration at pH 5.0-7.0 and 23 C. AS = alkyl sulfate.

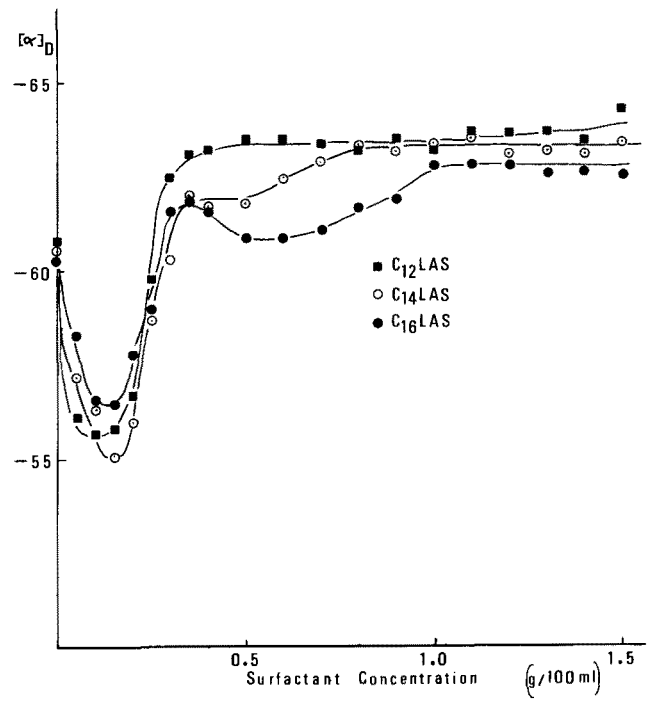


FIG. 2. The specific rotation of bovine serum albumin (BSA) as a function of surfactant concentration at pH 5.0-7.0 and 23 C. LAS = alkyl benzene sulfonate.

Specific rotation was calculated by applying the optical rotation to the equation: $[\alpha]_D = 100 \times \alpha / d \times c$ (I), where α, d, and c are optical rotation, cell length (dm), and BSA concentration, respectively.

To confirm the accuracy of the $\Delta[\alpha]_D$ value, defined as the difference between $[\alpha]_D$ values at 1.5g/100ml of surfactant concentration and in the absence of surfactants, the measurements were carried out at 1.5g/100ml of $C_{12}AS$ solution, and the standard deviation of $\Delta[\alpha]_D$ values was calculated from the 11 experiments. The standard deviation was 0.321, in terms of $\Delta[\alpha]_D$. Calculation of the least significant difference showed that in these experiments, the $\Delta[\alpha]_D$ values must have differed by 1.28 before they can be considered significantly different ($P<0.05$).

RESULTS AND DISCUSSION

Specific rotation, which is almost uninfluenced by pH changes between 4.5-7.0, was measured without using any buffer and plotted against the surfactant concentrations. The results obtained by using various anionic surfactants, AS, LAS, and AOS, are shown in Figures 1, 2, and 3, respectively. In the case of most anionic surfactants, $[\alpha]_D$ values changed with the concentrations in the following patterns. Initially, with the addition of a small amount of surfactants, the $[\alpha]_D$ value rapidly decreased in levorotation to a minimum, then gradually increased with an increase in the concentration, and finally reached a plateau value at a concentration of 1.0-1.5g/100ml.

The interaction between BSA and $C_{12}AS$ was shown to proceed by a stepwise mechanism (12,13). At relatively lower concentrations, the surfactant bound with cationic sites located on the surface of BSA and fixed, rather than unfolded, the 3-dimensional conformation of the BSA molecule, accompanying a decrease of levorotation in $[\alpha]_D$. When these sites were saturated with an increase of surfactant concentration, the BSA molecule underwent a large conformational change, and the $[\alpha]_D$ value increased in levorotation to a plateau value. All the results obtained here from the anionic surfactants indicated almost the same tendency as described above.

On the other hand, Figure 4 shows the results for the

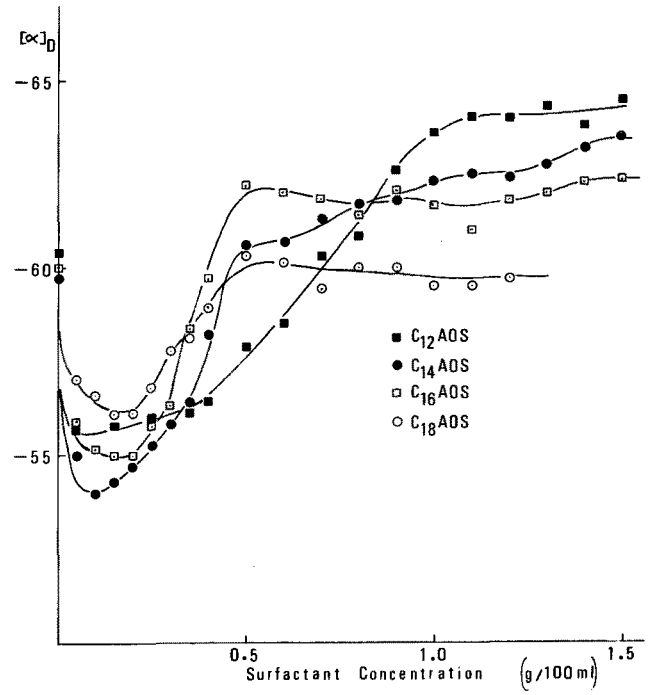


FIG. 3. The specific rotation of bovine serum albumin (BSA) as a function of surfactant concentration at pH 5.0-7.0 and 23 C. AOS = alpha-olefin sufonates.

cationic surfactants, C_{12}-, C_{14}-, and C_{16}TAC; in analogy to the case of anionic surfactants, the initial decrease in $[\alpha]_D$ value toward a minimum also were observed, but their extents were very small. Moreover, $[\alpha]_D$ value increased rapidly with increasing concentration, and at the concentrations of 0.5-1.5g/100ml, it reached a plateau value of which magnitudes were larger than those for anionic surfactants, especially in the case of $C_{12}TAC$. The results for the amphoteric surfactants, SB-12, SB-14, and B-12, are shown in Figure 5. The $[\alpha]_D$ curve for B-12 indicated a small increase in $[\alpha]_D$ toward a plateau, where-

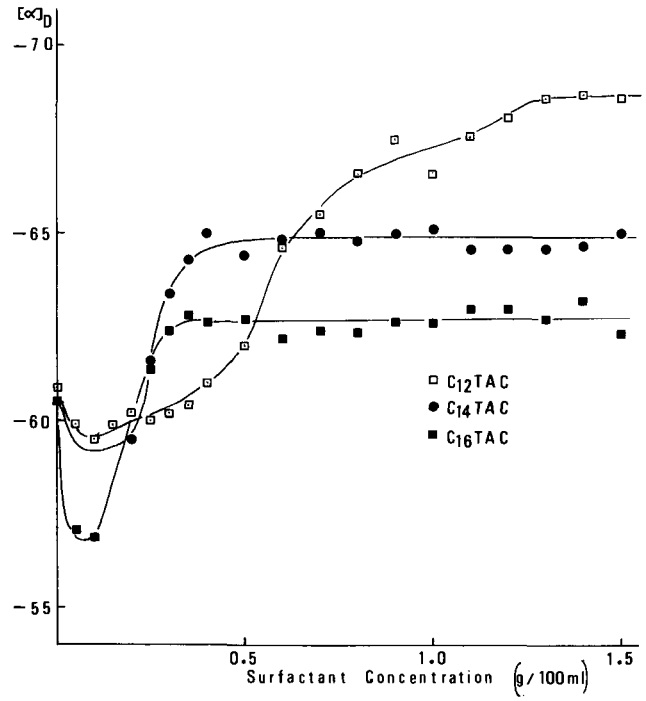


FIG. 4. The specific rotation of bovine serum albumin (BSA) as a function of surfactant concentration at pH 5.0-7.0 and 23 C. TAC = alkyl trimethyl ammonium chloride.

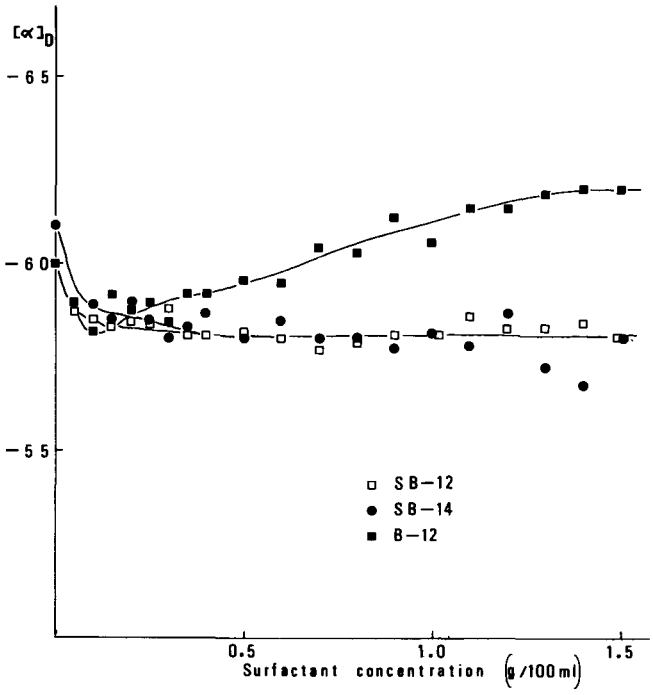


FIG. 5. The specific rotation of bovine serum albumin (BSA) as a function of surfactant concentration at pH 5.0-7.0 and 23 C. SB = alkyl sulfo betain; B = alkyl betain.

as those for SB-12 and SB-14 showed an initial small decrease at the low concentration, and no significant change with the increasing concentration. Figure 6 shows the results for the nonionic surfactants, C_{12}-7, C_{12}-8, and C_{12}-14EO, and also illustrates that although $[\alpha]_D$ curves showed an initial decrease at the low concentration, the values were almost independent of further increasing surfactant concentrations.

It can be pointed out (14) on the interaction between BSA and ionic surfactants, that the surfactants initially bound with the oppositely charged ionic groups on the surface of BSA, and when these sites were saturated with a certain binding amount of the ionic surfactants, a large conformational change in BSA molecule was induced. Thereby, the surfactant ions bound further with the new binding sites produced by the unfolding, leading to more complete unfolding, namely denaturation.

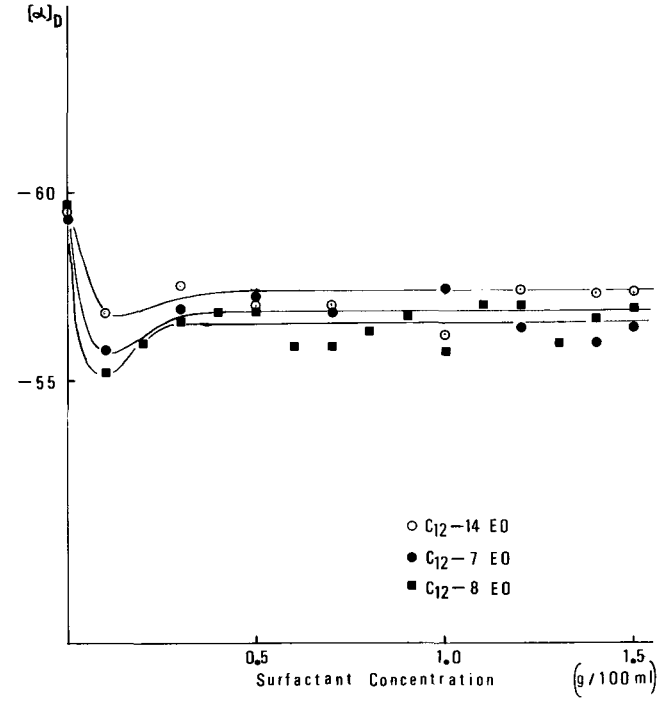


FIG. 6. The specific rotation of bovine serum albumin (BSA) as a function of surfactant concentration at pH 5.0-7.0 and 23 C. EO = alkyl polyoxyethylene.

It also was shown by the electrophoretic analysis (15,16) that the combination between BSA and C_{12}AS resulted in the formation of 3 discrete complexes having the approximate compositions, AS_{12}, AS_{55}, and AS_{110}, in response to varying surfactant-BSA ratios, where A and S represented BSA and C_{12}AS, respectively, and the numbers showed binding moles of surfactant to BSA. Each surfactant concentration at which the minimum in the $[\alpha]_D$ curve appeared and the $[\alpha]_D$ value attained a similar value to that of native BSA, corresponded to the first complex, AS_{11} in which the BSA molecule was fixed with adsorption of surfactants on the surface and the second complex, AS_{55}, in which the BSA molecule had about the same conformation as the native one. Moreover, the concentration at which the $[\alpha]_D$ curve became the plateau corresponded to the third complex, AS_{110}, in which the BSA molecule underwent a large denaturation. When the amount of the binding surfactants exceeded 110 moles to 1.0 mole of BSA, the additional changes in $[\alpha]_D$ scarcely were observed. In the case of the cationic surfactant such as C_{12}TAC, the BSA molecule was known first to undergo unfolding in the formation of the complex, AS_6 (17). On the other hand, it was indicated (18) with the interaction between BSA and nonionic surfactants that no significant change in BSA conformation brings out even in the presence of excess surfactants.

Therefore, at the concentration of 1.5g/100ml in which the amount of surfactant bound with 1.0 mole of BSA was calculated to be ca. 250 mole in the case of most surfactants and BSA molecule was completely denatured, the resulting $\Delta[\alpha]_D$ value, which represented the difference between $[\alpha]_D$ value at 1.5g/100ml concentration and in the absence of surfactants, was regarded as a measure of the protein denaturing potency. The $\Delta[\alpha]_D$ values for the various surfactants were determined and are summarized in

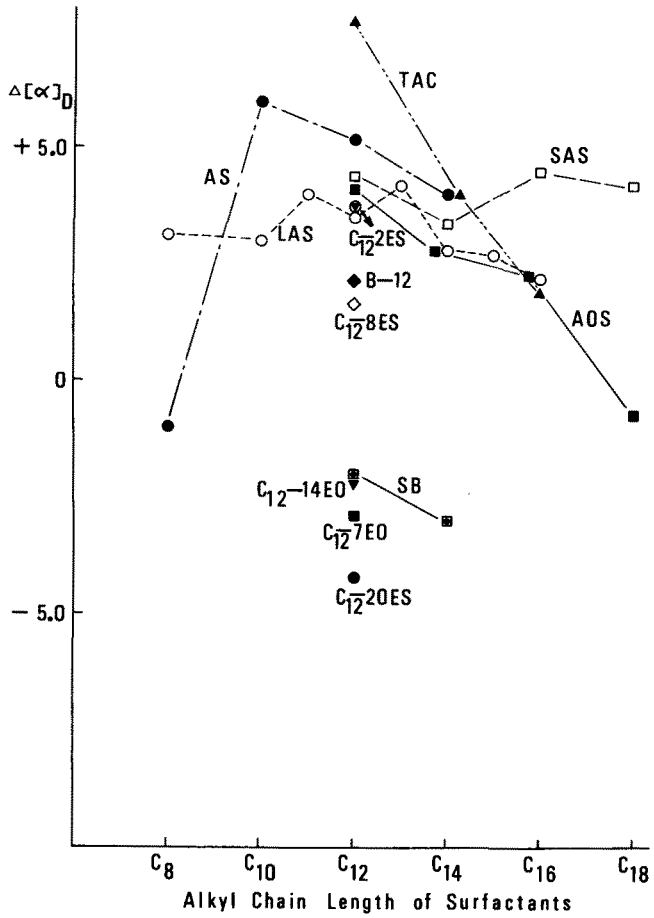


FIG. 7. The values of $\Delta[\alpha]_D$ obtained by various surfactants at 1.5g/100ml concentration. TAC = alkyl trimethyl ammonium chloride; SAS = paraffin sulfonate; LAS = alkyl benzene sulfonate; EO = alkyl polyoxyethylene; AOS = alpha-olefin sulfonate; SB = alkyl sulfo betain; ES = alkyl polyoxyethylene sulfate.

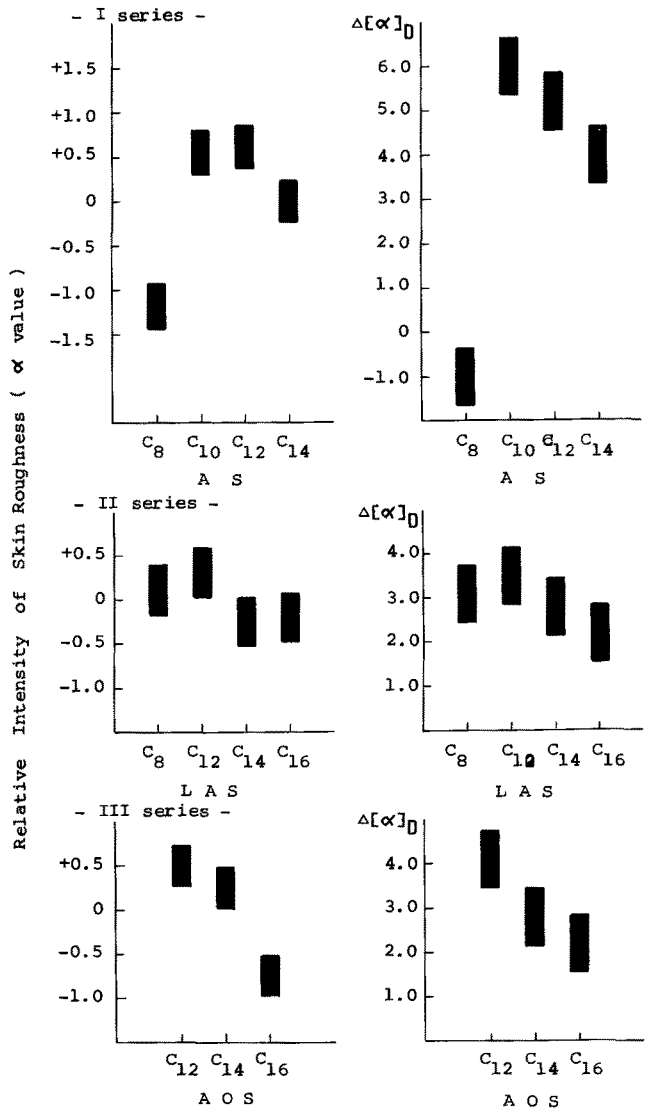


FIG. 8. Comparison between relative intensity of skin roughness (left figure) and bovine serum albumin (BSA) denaturing potency (right figure). AS = alkyl sulfate; LAS = alkyl benzene sulfonate; AOS = alpha-olefin sulfonate.

Figure 7. It was clear as seen in Figure 7 that $\Delta[\alpha]_D$ values were dependent on the alkyl chain length of surfactants, especially in the case of AS, AOS, and TAC, and that the $\Delta[\alpha]_D$ values of these series generally reached maximum when each of alkyl groups had 12 carbons.

These facts suggested that the interaction between BSA and the surfactants involved electrostatic attraction between oppositely charged ionic groups, as well as mutual association of nonpolar residues. Moreover, it can be pointed out that the surfactants, C_{12}TAC, C_{12}AS, and C_{10}AS, exhibited large denaturing potency, while the surfactants, C_{12}-20ES, C_{12}-7EO, C_{12}-14EO, SB-12, and SB-14 had small values.

Comparison between Skin Roughness and Protein Denaturation

If skin roughness is the result of denaturation of epidermal protein, it may be expected that there are some relationships between the abilities of surfactants to denature the protein and to roughen the skin. Therefore, the relationship between them was examined by comparing both the magnitude of the $\Delta[\alpha]_D$ values and the relative intensity of the skin roughness. The results for skin roughness were obtained by using the circulation method (11) in C_8, C_{10}, C_{12}, C_{14}-AS, (series I), C_8, C_{12}, C_{14}, C_{16}-LAS (series II), and C_{12}, C_{14}, C_{16}-AOS, (series III), and C_{12}AS, C_{12}LAS, C_{12}AOS, C_{12}SAS (system IV), C_{12}AS, C_{16}AOS, C_{16}LAS (system V), C_{12}AS, C_{12}-14EO, C_{12}-2ES, C_{12}Soap (system VI), and C_{12}AS, LAS, AOS, ES, (system VII) and those published already by Okamoto (19) using the immersion and the dropping methods in LAS, SAS, ES (system VIII), and B-12, LAS, ES, C_{12}-7EO (system IX) also were used.

The comparisons for series and systems are shown in Figures 8,9,10. In Figure 8, series I indicated that the intensities of skin roughness decreased in the order of $C_{12}AS \geqslant C_{10}AS > C_{14}AS > C_8AS$ with a significant difference at the probability level of 95%, while $\Delta[\alpha]_D$ values for the same series were in the order of $C_{10}AS \geqslant C_{12}AS \geqslant C_{14}AS > C_8AS$. Both results showed good agreement with each other. The other series and systems, II through VI, illustrated that the orders of their intensities of skin roughness were: $C_{12}LAS \geqslant C_8LAS \geqslant C_{16}LAS \geqslant C_{14}LAS$ in series II; $C_{12}AOS \geqslant C_{14}AOS > C_{16}AOS$ in series III; $C_{12}AS \geqslant C_{12}AOS \geqslant C_{12}SAS \geqslant C_{12}LAS$ in system IV; $C_{12}AS > C_{16}AOS \geqslant C_{16}LAS$ in system V; and $C_{12}Soap > C_{12}AS \geqslant C_{12}$-2ES $\geqslant C_{12}$-14EO in system VI, at the same probability level. They were in fair agreement with those of the magnitudes of $\Delta[\alpha]_D$: $C_{12}LAS \geqslant C_8LAS \geqslant C_{14}LAS \geqslant C_{16}LAS$ in series II; $C_{12}AOS > C_{14}AOS \geqslant C_{16}AOS$ in series III; $C_{12}AS \geqslant C_{12}SAS \geqslant C_{12}AOS \geqslant C_{12}LAS$ in system IV; $C_{12}AS > C_{16}AOS \geqslant C_{16}LAS$ in system V, and $C_{12}AS > C_{12}$-2ES $> C_{12}$-14EO in system VI. Only in the VI system was it impossible to determine the $\Delta[\alpha]_D$ value for C_{12}Soap because of turbidity and strong alkalinity of the

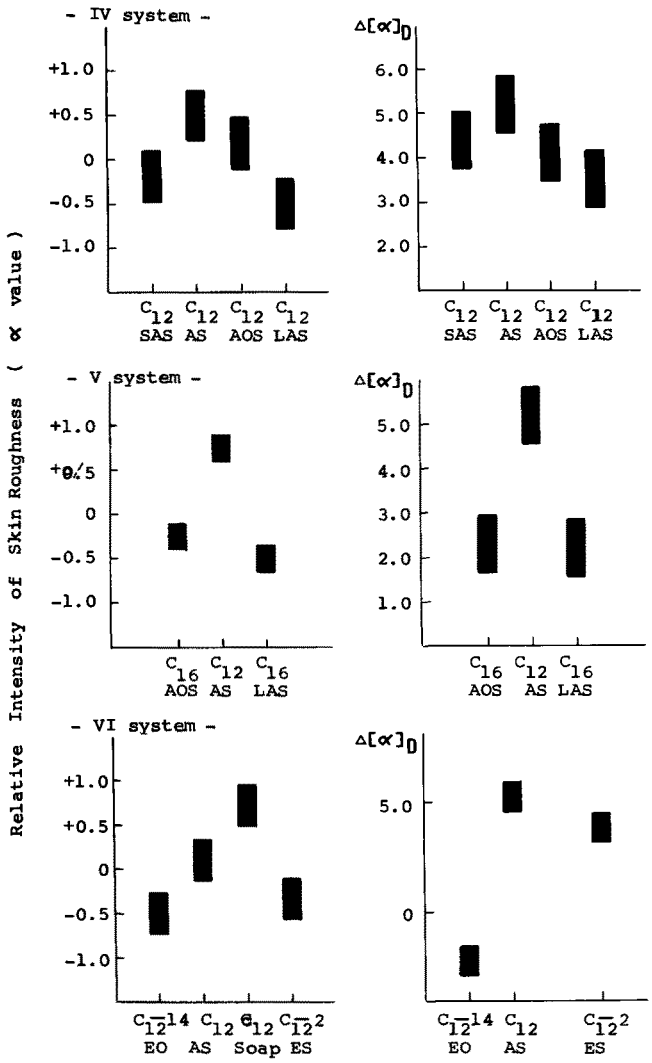


FIG. 9. Comparison between relative intensity of skin roughness (left figure) and bovine serum albumin (BSA) denaturing potency (right figure). SAS = paraffin sulfonate; AS = alkyl sulfate; AOS = alpha-olefin sulfonate; LAS = alkyl benzene sulfonate; EO = alkyl polyoxyethylene; ES = alkyl polyoxyethylene sulfate.

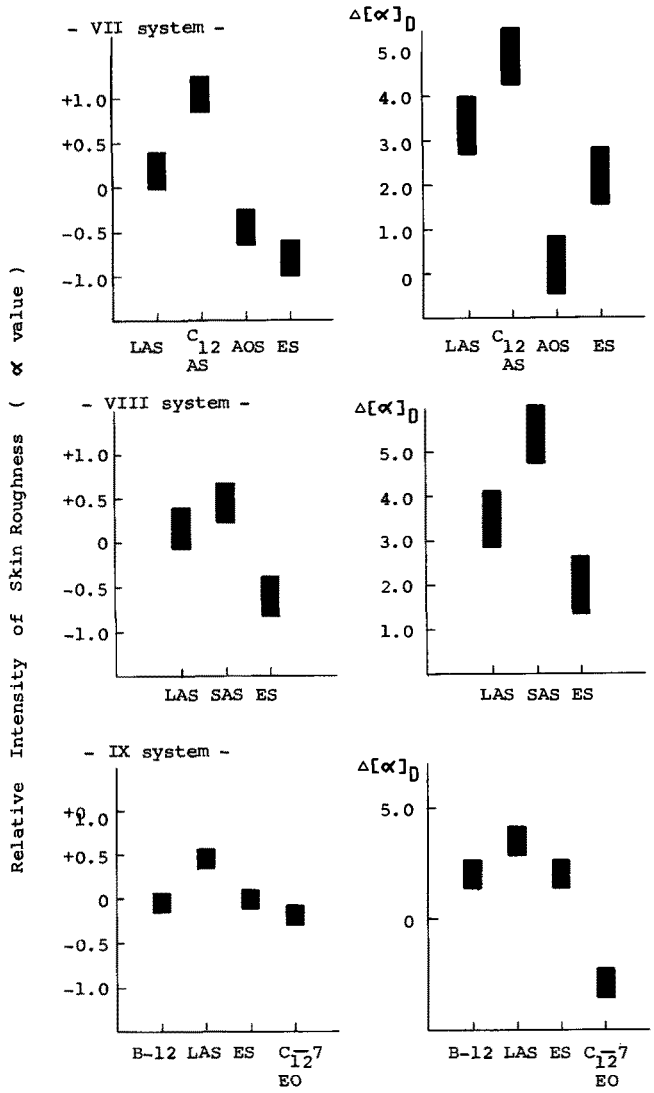


FIG. 10. Comparison between relative intensity of skin roughness (left figure) and bovine serum albumin (BSA) denaturing potency (right figure). LAS = alkyl benzene sulfonate; AS = alkyl sulfate; AOS = alpha-olefin; ES = alkyl polyoxyethylene sulfate; SAS = paraffin sulfonate; EO = alkyl polyoxyethylene.

solution. Therefore, comparison only among C_{12}AS, C_{12}-14EO, and C_{12}-2ES was performed.

It was important to examine whether the correlation observed above is applicable for commercial detergents or not. In confirmation of this fact, the comparisons for 3 systems, VII, VIII, and IX, were made as shown in Figure 10, where $\Delta[\alpha]_D$ values for these detergents, except B-12 and C_{12}-7EO, were determined only at 1.5g/100ml of the surfactant concentration. The orders obtained from both their intensities and magnitudes were: C_{12}AS > LAS > AOS ≥ ES in system VII; SAS ≥ LAS > ES in system VIII; and LAS > ES ≥ B-12 ≥ C_{12}-7EO in system IX; and C_{12}AS > LAS ≥ ES > AOS in system VII; SAS > LAS > ES system VIII; and LAS > ES ≥ B-12 > C_{12}-7EO in system IX. Thus, they also showed good agreement with each other.

As already described (20) it is known that with the interaction between BSA and surfactants the first process is a reversible ionic adsorption of surfactant onto protein, and that a sufficient amount of adsorbing surfactants is required for denaturation. Therefore, it is highly probable that the surfactant with a powerful adsorbing action also have a pronounced denaturing ability for protein.

On the basis of these facts, the remarkable correlation obtained between the BSA-denaturing potency and skin roughness might be explained by assuming that the adsorption of surfactants onto skin may play an important role in roughening the skin. Our previous work (21) also had indicated that there is an apparent correlation between the roughening effect of surfactants on skin and its adsorption onto isolated callus used as a representative of epidermal keratin of human skin.

In addition, this technique using the specific rotation of BSA is easily applicable to evaluating more than 20 surfactants at the same time under various conditions at pH 4.5-7.0, and it also is useful in predicting the skin roughening potency of surfactants without using human skin.

ACKNOWLEDGMENTS

K. Nagase, Director of Research Laboratories, encouraged this work and permitted publication of this paper. T. Sato and K. Uenoyama helped in this work.

REFERENCES

1. Blench, W., Fette Seifen Anstrichm. 63:622 (1961).
2. Kirk, J.F., Acta. Derm. Vener. 46(Suppl 57):24 (1966).
3. Blank, H., J. Invest. Derm. 25:391 (1955).
4. Van Scott, E.J., and J.B. Lyon, Ibid. 21:199 (1953).
5. Harrold, S.P., Ibid. 32:581 (1959).
6. Czok, R., Surf. Cong. 5:337 (1969).

7. Lansdown, A.B.G., Brit. J. Derm. 86:361 (1972).
8. Jirgensons, B., Arch. Biochem. Biophys. 39:261 (1952).
9. Doty, P., J. Amer. Chem. Soc. 79:761 (1957).
10. Doty, P., U. Polymer Science 23:851 (1957).
11. Imokawa, G., K. Sumura, and M. Katsumi, JAOCS 52:479 (1975).
12. Yang, J.T., and J.F. Foster, J. Am. Chem. Soc., 75:5560 (1953).
13. Pallansch, M., and D.R. Briggs, Ibid. 76:1396 (1954).
14. Putnan, F.W., Advan. Protein Chem. 4:79 (1948).
15. Putnan, F.W., and H. Neurath, J. Boil. Chem. 159:195 (1945).
16. Aoki, K., J. Am. Chem. Soc., 80:4904 (1958).
17. Few, A.V., R.H. Ottevill, and H.C. Perreia, Biochim. Biophys. Acta. 18:136 (1955).
18. Green, F.A., J. Colloid. Interface Sci. 35:481 (1971).
19. Okamoto, T., J. Jap. Oil Chem. Soc., 21:151 (1972).
20. Reynolds, J.A., and S. Herbert, Biochemistry, 6:937 (1967).
21. Imokawa, G., and Katsumi, M., J. Jap. Oil Chem. Soc. 23:719 (1974).

[Received April 25, 1974]