• **Technical**

Study on Skin Roughness Caused by Surfactants: I. A New Method In Vivo for Evaluation of Skin Roughness

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ABSTRACT

A simple and accurate method for evaluating skin roughness caused by surfactants has been developed. The intensities of the roughness have been correlated with the chemical structures of surfactants by using this method. Results indicated in general, that the surfactants having 12 carbons in their alkyl chain gave the skin more roughness than did those which had more or less than 12. The results obtained by this method were in good agreement with those from the *immersion method* usually used.

INTRODUCTION

Production of dishwashing detergents containing synthetic surfactants as a main ingredient has increased annually, and most detergents have been consumed for household use. In principle, dishes are washed 3 times a day for more than 10 minutes at a time, causing repeated skin contact with detergent solutions. Many consumers have claimed that hand roughness has been caused by the use of the detergents.

It is worthwhile to eliminate skin problems which may be due to detergent, and, therefore, it is important to make detergents which have a mild effect on skin. In an attempt to develop mild detergents, it was necessary to examine the relationship between the chemical structures of surfactants and the intensities of skin roughness. This attempt needed a simple and accurate in vivo method that would satisfy the following requirements. It needed as small number of human subjects and amount of surfactant as possible; it needed to be pleasant and not difficult for human subjects; and it needed to reproduce skin roughness with high accuracy.

Although the immersion method usually used was the best simulation of actual use, it did not satisfy the above requirements. From this viewpoint, a technique called the circulation method has been developed in this paper. In this method, the inner surface of the forearm was roughened by contact with surfactant solutions which were circulated through a specially designed apparatus.

Although numerous investigations have been reported on skin roughness with surfactants, most surfactants used in the experiments were commercial products which contained various compositions of raw materials and impurities, and it was doubtful whether the skin roughness obtained was caused by the surfactant. Consequently, to investigate the influence of the chemical structure of surfactants on the skin roughness, it was necessary to use samples in which purities, isomeric ratios, and alkyl distributions were determined.

The objective of this paper was to develop a new method for quantitatively evaluating the skin roughness caused by surfactants whose structures were determined distinctly. Simultaneously, the correspondence of the results obtained by the circulation method to those by the immersion method was examined for the purpose of confirming the

TABLE I

Analytical Data of Surfactants

Surfactant[a]	Distribution of alkyl chain length (%)	Purities[b] (%)
C_8 AS	C_8: 99.80	97.72
C_{10}AS	C_{10}: 99.9 C_{12}: 0.1	97.17
C_{12}AS	C_{12}: 100	100
C_{14}AS	C_{14}: 99.8	99.3
C_8 LAS	C_8 : 100	98.5
C_{10}LAS	C_{10}: 100	98.7
C_{11}LAS	C_{11}: 100	97.6
C_{12}LAS	C_{12}: 100	97.88
C_{13}LAS	C_{13}: 100	99.4
C_{14}LAS	C_{14}: 100	97.6
C_{15}LAS	C_{15}: 100	99.2
C_{16}LAS	C_{16}: 100	97.40
C_{12}AOS	C_{12}: 99.97 C_{14}: 0.03	96.00
C_{14}AOS	C_{14}: 100	88.80
C_{16}AOS	C_{16}: 100	90.76
C_{18}AOS	C_{18}:100	88.09
B-12	---	90.32
B-16	---	95.94
SB-12	---	99.03
SB-14	---	99.90
C_{12}SAS	C_{12}: 100	95.3[b)]
C_{14}SAS	C_{14}: 100	96.4[b)]
C_{16}SAS	C_{16}: 100	96.8[b)]
C_{18}SAS	C_{18}: 100	97.5[b)]

[a]AS = alkyl sulfate; LAS = alkyl benzene sulfonate; AOS = alphaolefin sulfonate; B = alkyl betain; SB = alkyl sulfo betain; SAS = paraffin sulfonate.

[b]Determined by the data on elemental analysis. Determined by Epton's method.

TABLE II

Ratios of the Isomer of Alkylbenzene Sulfonate

Surfactant[a]	2 Ph[b] (%)	3 Ph (%)	4 Ph (%)	5 Ph (%)	$\geq$6 Ph (%)
C_8 LAS	48.90	27.60	23.50		
C_{10}LAS	42.80	23.10	16.70	17.40	
C_{11}LAS	35.51	22.40	17.42	24.67	
C_{12}LAS	31.10	18.04	15.83	35.02	35.02
C_{13}LAS	33.85	20.48	13.86	13.76	18.05
C_{14}LAS	31.81	18.03	12.91	14.26	22.99
C_{15}LAS	28.85	16.39	12.51	12.02	30.24
C_{16}LAS	30.33	17.62	11.54	10.97	29.54

[a]LAS = alkyl benzene sulfonate.

[b]X Pheny shows percent of benzene group binding at x number carbons from terminal alkyl carbon.

TABLE III

Ratios of Alkene and Hydroxy Sulfonate in Alpha-Olefin Sulfonate (AOS)

Surfactant	Alkene (%)	Hydroxy (%)
$C_{12}AOS$	78.3	21.7
$C_{14}AOS$	70.7	29.3
$C_{16}AOS$	73.9	26.1
$C_{18}AOS$	74.3	25.7

TABLE IV

Ratios of Mono- and Di-Paraffin Sulfonate (SAS)

Surfactant	Mono- (%)	Di- (%)
$C_{12}SAS$	97.0	3.0
$C_{14}SAS$	91.7	8.3
$C_{16}SAS$	97.4	2.6
$C_{18}SAS$	97.0	3.0

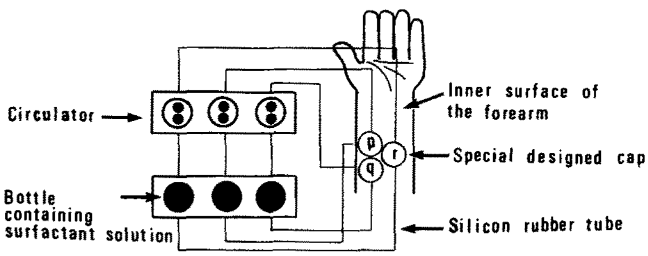

FIG. 1. Specially designed apparatus for circulation method.

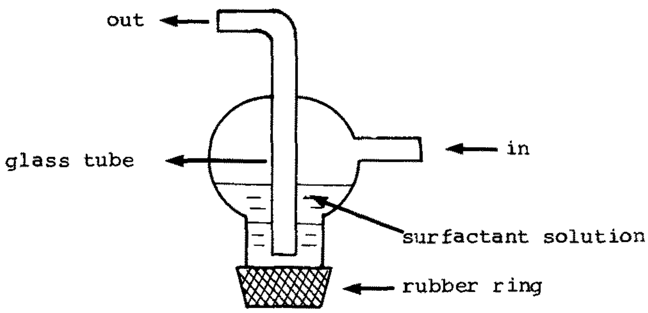

FIG. 2. Specially designed cap.

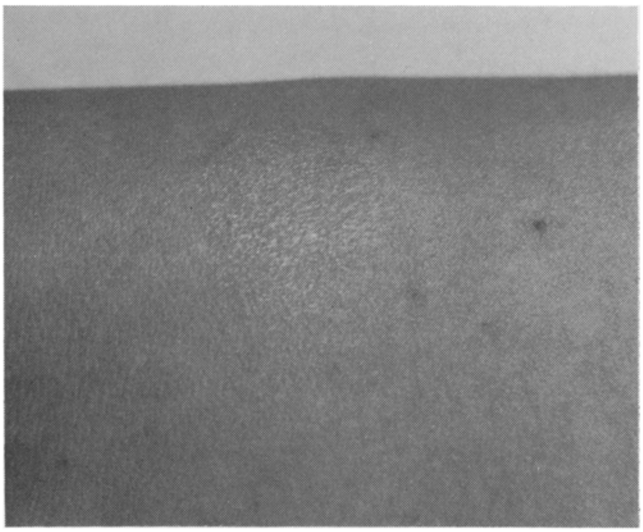

FIG. 3. Abnormal scaling and dryness of skin caused by circulation method.

applicability of the circulation method.

EXPERIMENTAL PROCEDURES

Materials

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 ($C_nH_{2n+1}C_6H_4SO_3Na$ [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 (soap) ($C_{n-1}H_{2n-1}$ 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]) were prepared by the usual synthetic method.

These products, except C_nSoap, B-n, SB-n, and C_n-pEO, were dissolved in 80-90% (w/w) ethanol aqueous solution and filtered to remove inorganic salts. After diluting to ca. 50% solution with water, each unsulfonated or unsulfated material was extracted with petroleum ether. B-n and SB-n were recrystallized 3 times from ethanol, and C_n-pEO was purified by removing polyethylene glycol.

The purities of the specimens were determined by elemental analysis or Epton's method (1). Alkyl sulfate (AS) was decomposed by reaction with sulfuric acid, and then the alkyl distribution was determined by gas chromatography from the resulting alcohol. Both alkyl distributions of AOS and SAS were also determined by the same technique from their original materials, namely alfaolefin and paraffin. Alkyl distribution and the ratio of isomers in LAS were determined by Knight's method (2). Ratios of alkenyl and hydroxy form in AOS and of mono- and di- components in SAS also were determined by Konishi's method (3) and Mutter's method (4), respectively. The data obtained are summarized in Table I, II, III, and IV.

METHODS

Skin Roughness Test – Circulation Method

As Figure 1 shows, 3 glass caps were fixed on the inner surface of the forearm. Then, these caps, 3 bottles containing 150 ml of 1.0% surfactant solution, and a circulator equipped with 3 pumps were connected with silicon rubber tube. To cause skin roughness on regions occupied by the caps, 150 ml each of 3 kinds of surfactant solutions in concentration of 1.0% were circulated through the apparatus at the same time with a constant flow rate of 200 ml/min at 40 C for 10 min. Although 1.0% of surfactant concentration was higher than that usually used privately, it was suitable for determining more distinctly the relative intensities of the skin roughness among various surfactants. After this treatment, the arm was washed 3 times with water by immersing it in a bath. This test was carried out daily for 3 or 4 days, successively. Twenty-four hours after the last treatment, the difference of skin roughness among the 3 areas on the forearm were evaluated and compared to each other on the basis of criteria described later. All above processes were done under double blind conditions. Eight subjects participated in comparing 4 surfactants, and 6 subjects participated in comparison of 3 surfactants.

As seen in Figure 2, the special cap, which had a glass tube in it, was designed to prevent foaming due to mixing of air during circulation. In addition, a rubber ring was attached at the bottom of the cap to provide close contact between the cap and the skin.

Statistical Evaluation of Skin Roughness

After treatment by the circulation method, the resulting

TABLE V

Results of Skin Roughness Test by Circulation Method[b]

	Subject Number[b]							
Surfactants	1	2	3	4	5	6	7	8
$C_{12}AS$-$C_{12}LAS$	+1	+1	+2	-1				
$C_{12}AS$-$C_{12}AOS$	+1	+1					+1	0
$C_{12}AS$-$C_{12}SAS$			+1	-1			+1	+1
$C_{12}LAS$-$C_{12}AS$	-1	-1	-2	+1				
$C_{12}LAS$-$C_{12}AOS$	0	0			-2	-1		
$C_{12}LAS$-$C_{12}SAS$			-1	0	-1	0		
$C_{12}AOS$-$C_{12}AS$	-1	-1					-1	0
$C_{12}AOS$-$C_{12}LAS$	0	0			+2	+1		
$C_{12}AOS$-$C_{12}SAS$					+1	+1	0	+1
$C_{12}SAS$-$C_{12}AS$			-1	+1			-1	-1
$C_{12}SAS$-$C_{12}LAS$			+1	0	+1	0		
$C_{12}SAS$-$C_{12}AOS$					-1	-1	0	-1

[a]LAS = alkyl benzene sulfonate; AOS = alpha-olefin sulfonate; SAS = paraffin sulfonate; AS = alkyl sulfate.

[b]In $C_{12}AS$, $C_{12}LAS$, $C_{12}AOS$ and $C_{12}SAS$ system, where, for example in subject No.1 the 3 furfactants, $C_{12}AS$, $C_{12}LAS$, and $C_{12}AOS$, were tested at the same time, and comparisons of intensities of skin roughness were performed between $C_{12}AS$ and $C_{12}LAS$, $C_{12}AS$ and $C_{12}AOS$, and $C_{12}AOS$ and $C_{12}LAS$.

TABLE VI

Results of Analysis of Variance[a]

	Sum of squares		Degrees of freedom	Mean square	F_o	$F_{0.05}$	$F_{0.01}$
	Symbol	Value					
Main effects	$S\alpha$	18.25	3	6.083	8.42	2.92	4.51
Deviations from subtractivity	S_r	3.75	3	1.250	1.73		
Order effects	S_δ	0	6	---	---		
Error	S_ϵ	26	36	0.722			

$Y_{0.05} = 0.576$

[a]In C_{12}alkyl sulfate (AS), C_{12}alkyl benzene sulfonate (LAS), C_{12}alpha-olefin paraffin (AOS), and C_{12}paraffin sulfonate (SAS) system where $Y_{0.05}$ was calculated according to the equation: $Y_{0.05} = q_{0.05}\sqrt{V\epsilon / 2\,rm}$ V_ϵ = mean square for error; r = number of judge; m = number of brand (surfactant) to be compared; $q_{0.05}$ was found to be 3.84 by examining the table of the Studentized range for a range of m=4 variates and for ϕ=36 degrees of freedom.

skin response was characterized mainly by gross visible changes, such as abnormal scaling and dryness of the upper layer of skin (Fig. 3), referred to here as skin roughness. Primary irritation, such as erythema and edema scarcely was observed. In a few cases, slight erythema was invoked soon after the surfactant treatment was terminated. In addition, treatment by water only under the same conditions induced no visible changes on skin.

The relative intensities of skin roughness were evaluated by using the following criteria based on the intensity of the scaling: +2 – i was strongly scaling in comparison with j; +1 – i was slightly scaling in comparison with j; 0 – no difference between i and j; -1 – j was slightly scaling in comparison with i; -2 – j was strongly scaling in comparison with i; where i and j were a pair to be compared. For example, if intensities of the resulting skin roughness was observed among the 3 areas, p, q, and r, in the order of $p > q > r$ or $p = q > r$ as described in Figure 1, the following points were given; p,q) = +1, (p,r) = +2, (q,r) = +1, or (p,q) = 0, (p,r) = +1 or +2, (q,r) = +1 or +2.

The data obtained by the above evaluation were analyzed statistically by Scheffe's paired comparison method (5). Relative intensities of skin roughness were expressed as α values of the main effects after a significant difference was confirmed at the 99% or 95% probability level, and yard stick, $Y_{0.05}$ or $Y_{0.01}$, was calculated from the results of an analysis of variance for the purpose of examinating all the significant differences among their obtained main effects.

It was important to make sure that there was no difference in skin reactivity among the 3 areas of the forearm. Therefore, a preliminary experiment was carried out by using the same solution of 1.0% $C_{12}AS$ under the same conditions as described above. Results revealed that there was no difference of skin reactivity among the 3 areas. To give further reliability for this method, all of the tests were designed as a balanced incomplete block (6).

RESULTS

The skin roughness test by the circulation method has been carried out using 4 systems and 3 series of surfactants, such as $C_{12}AS$-$C_{12}LAS$-$C_{12}AOS$-$C_{12}SAS$ (I), $C_{12}AS$-$C_{16}AOS$-$C_{16}LAS$ (II), $C_{12}AS$-$C_{12}AS$+SB-12-C_{12}8ES (III), and $C_{12}AS$-C_{12}14EO-C_{12}-2ES-C_{12}Soap (IV) system, and C_8AS-$C_{10}AS$-$C_{12}AS$-$C_{14}AS$ (V), C_8LAS-$C_{12}LAS$-$C_{14}LAS$-$C_{16}LAS$ (VI), and $C_{12}AOS$-$C_{14}AOS$-$C_{16}AOS$ (VII) series.

Primarily Table V shows the results of the skin roughness test for system I. The data obtained were analyzed statistically by Scheffe's method. The main effects of each surfactant were calculated to be +0.500, -0.500, +0.188, and -0.188, respectively, where (+) indicated the tendency to be more roughening, and (-) indicated less roughening. Moreover, the results of analysis of variance, as shown in Table VI, indicated that there was a significant difference ($P<0.05$) and $Y_{0.05}$ also was calculated to be 0.576.

These findings are shown in Figure 4-1 which indicate

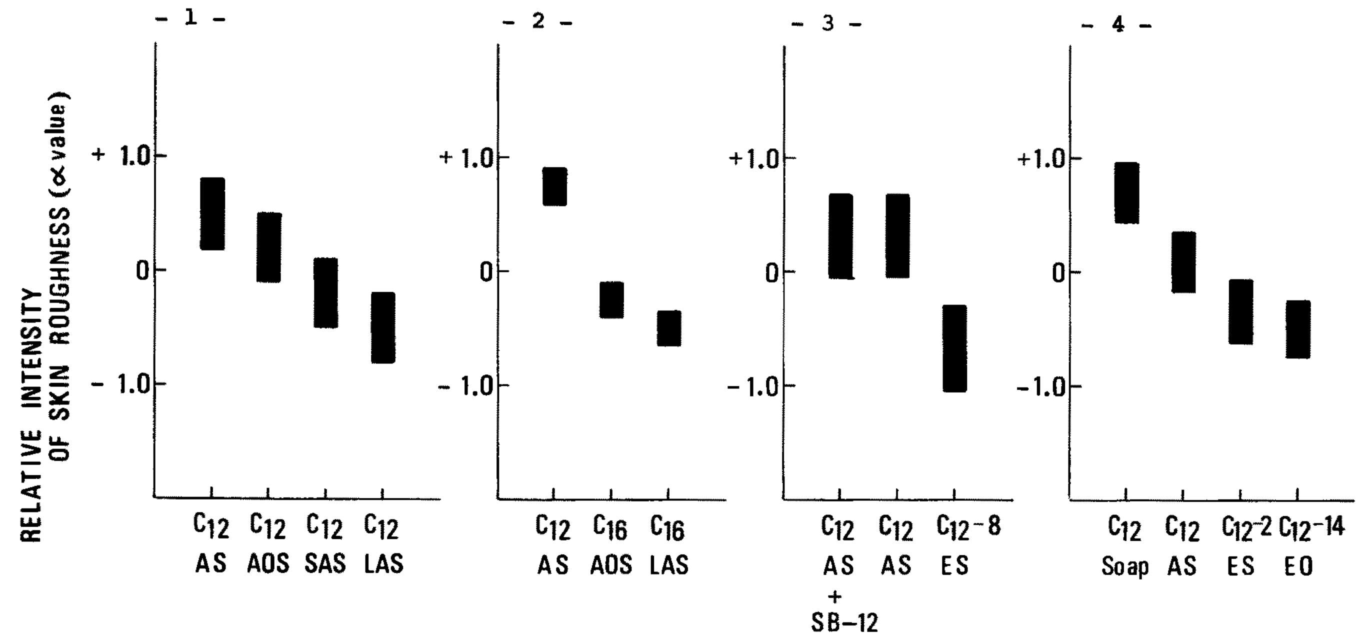

FIG. 4. Relative intensities of skin roughness obtained by circulation method in 4 different groups of surfactants. AS = alkyl sulfate; AOS = alpha-olefin sulfonate; SAS = paraffin sulfonate; LAS = alkyl benzene sulfonate; ES = alkyl polyoxyethylene sulfate; EO = alkyl polyoxyethylene.

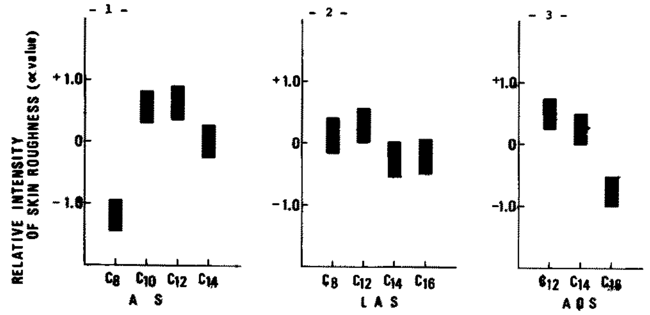

FIG. 5. Relative intensities of skin roughness by circulation method in 3 different groups of surfactants. AS = alkyl sulfate; LAS = alkyl benzene sulfonate; AOS = alpha-olefin sulfonate.

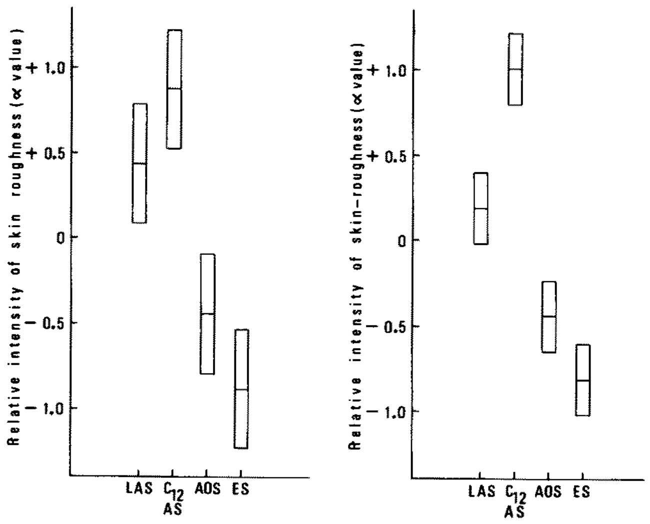

FIG. 6. Comparison of the circulation method with the immersion method in C_{12} alkyl sulfate (AS), alkyl benzene sulfonate (LAS), alpha-olefin sulfonate (AOS), and alkyl polyoxyethylene sulfate (ES) system, where the left and right figure indicate the results of the immersion method and the circulation method, respectively, and where block overlap difference between surfactants have no statistical significance at probability level of 95% in left figure and of 99% in right figure.

that roughness caused by each surfactant decreased on the order of $C_{12}AS \geqslant C_{12}AOS \geqslant C_{12}$ SAS $\geqslant C_{12}LAS$. There existed a significant difference with a confidence coefficient of 95% between each surfactant, except between $C_{12}AS$ and $C_{12}AOS$, $C_{12}AOS$ and $C_{12}SAS$, and $C_{12}SAS$ and $C_{12}LAS$, where the rectangles in figures mean fiducial limits responsible for the value of $Y_{0.05}$.

Secondly, the same tests were carried out for systems II, III, and IV, and series V, VI, and VII. Similar statistical calculations indicated that their main effects were +0.750, -0.250, and -0.500 in system II, +0.333, +0.333, and -0.666 in system III, +0.111, -0.500, -0.333, and +0.722 in system IV, -1.188, +0.563, +0.624, and 0.000 in series V, +0.125, +0.313, -0.250, and -0.188 in series VI, and +0.500, +0.250, and -0.750 in series VII. There was a significant difference ($P<0.05$ in all systems and series, and $Y_{0.05}$ was also calculated to be 0.300, 0.723, 0.468, 0.488, 0.560, and 0.458, respectively.

Therefore, as illustrated clearly in Figures 4 and 5, the intensities of roughness corresponding to each surfactant in each system and series were in the following orders: $C_{12}AS > C_{16}AOS \geqslant C_{16}LAS$ in system II; $C_{12}AS = C_{12}AS$+SB-12 $> C_{12}$-8ES in system III; $C_{12}Soap \geqslant C_{12}AS \geqslant C_{12}$-2ES $\geqslant C_{12}$-14EO in system IV; $C_{12}AS \geqslant C_{10}AS > C_{14}AS > C_8AS$ in series V; $C_{12}LAS \geqslant C_8LAS \geqslant C_{16}LAS \geqslant C_{14}LAS$ in series VI; and $C_{12}AOS \geqslant C_{14}AOS > C_{16}AOS$ in series VII.

Comparison of Circulation Method with Immersion Method

Okamoto (7) recently has reported that the intensity of skin roughness obtained by the immersion method was found to decrease in the order of $C_{12}AS \geqslant$ LAS $>$ AOS $\geqslant$ ES.

The skin roughness test by the circulation method has been performed by using the same group of detergents for a comparison of both methods. The results showed that their main effects were +1.063 ($C_{12}AS$), +0.188 (LAS), -0.438 (AOS), and -0.813 (ES), and $Y_{0.01}$ was 0.424. These data indicated that the intensity of the roughness decreased in the order of $C_{12}AS >$ LAS $>$ AOS $\geqslant$ ES with a significant difference ($P<0.01$) between each surfactant, except between AOS and ES. In comparison of both results as is clear from Figure 6, it was found that they were quite in agreement with each other in order of each surfactant.

DISCUSSION

The roughness test was carried out using 4 systems and 3 series of surfactants to confirm the practical applicability of the circulation method. The relative intensities of the roughness were expressed by the main effects (α value) with a confidence coefficient of 95% in all surfactant systems and series. This fact suggested that the circulation method had good accuracy, in spite of using a small number of human subjects.

From the results, it was found that C_{12}Soap and AS, especially C_{12}AS, had more roughening potential, and ES and EO had less roughening potential, and that the surfactants which had an alkyl chain length longer or shorter than 12, such as C_{16}AOS, C_{16}LAS, and C_8AS, were less roughening. These results were similar to the known facts (6,8) on the roughness caused by surfactants, except for the results with C_{12}Soap. For the further examination of the applicability, the roughness test by the circulation method was performed by using the same surfactant system studied by the immersion method. The results agreed completely with those obtained by the immersion method. These facts suggested that the circulation method developed here was applicable to the quantitative evaluation of skin roughness caused by surfactants.

Moreover, this method had the following advantages. Only a small number of human subjects and small amount of surfactants were needed to test the roughness. Roughened skin was uninfluenced by subjects usual activities until the diagnosis was finished, and evaluation of the resulting roughness was easy becuase the roughened region was limited to a part of the inner surface of the forearm, a region which is scarcely used by human subjects in daily routines.

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