TABLE V

Protein Results on Water-Dispersible Collaborative Study

	Type of Product				
	44% S.B.O.M.	Medium toasted flakes	Toasted grits	Light toasted flakes	Light toasted flour
Laboratory 1					
O1, B1, D1	15.0	18.4	14.3	39.7	39.7
D2	14.5	18.5	14.3	39.5	39.9
B2, D1	14.6	17.6	13.3	39.3	39.5
D2	14.0	17.1	13.5	39.2	39.5
O2, B1, D1	14.7	18.7	14.5	40.4	40.6
D2	15.0	18.2	14.8	39.7	40.0
B2, D1	14.2	18.2	13.9	39.9	40.1
D2	14.6	17.3	14.2	39.8	40.1
Laboratory 2					
O1, B1, D1	13.2	15.9	13.5	39.4	39.9
D2	13.7	16.1	13.4	38.8	39.0
B2, D1	12.8	14.6	12.8	39.0	39.4
D2	13.2	15.8	13.9	40.1	41.1
O2, B1, D1	13.6	15.4	15.2	39.4	39.5
D2	12.8	17.4	14.2	40.2	39.7
B2, D1	13.8	15.9	13.5	39.2	41.0
D2	13.3	16.0	14.0	40.7	41.0
Laboratory 3					
O1, B1, D1	14.1	16.9	14.1	39.7	39.4
D2	14.2	17.4	15.3	39.4	40.1
B2, D1	14.2	16.2	15.3	40.3	40.3
D2	13.7	15.9	14.5	39.1	39.9
O2, B1, D1	14.7	17.2	15.2	39.5	39.2
D2	14.4	16.8	15.0	39.5	39.7
B2, D1	14.2	16.7	14.8	38.8	37.8
D2	14.0	16.5	14.3	39.2	39.3
Average	14.0	16.9	14.2	39.6	39.8
Std. deviation	0.74	1.25	0.75	0.59	0.79

O—Operators, B—Blendors, D—Days. See experimental design, Figure 7.

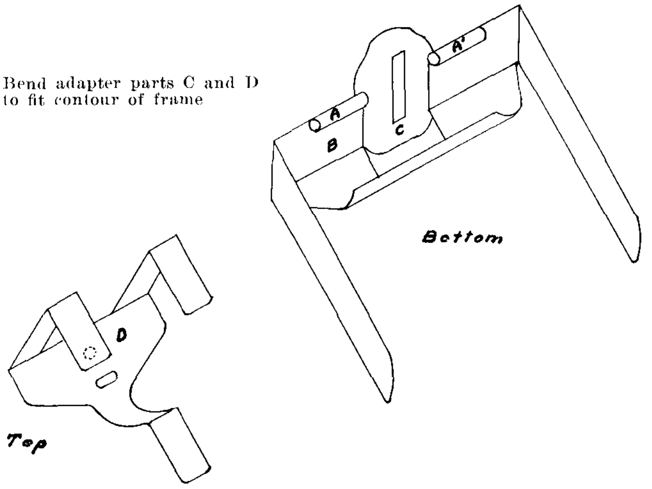
Bend adapter parts C and D to fit contour of frame

FIG. 9. Adapter assembly.

Summary

A method is presented for determination of Water-Dispersible Protein, using a Hamilton Beach Drinkmaster #30 modified to accommodate Waring Blendor blades and cups. The probable sources of error in the method are discussed, and the precision of the method is shown, based on the results obtained in three laboratories using six different Modified Hamilton Beach Blendors.

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Surface-Active Properties of Sodium Salts of Sulfated Fatty Acid Monoglycerides

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STUDIES ON THE SULFATION of fatty acid-glycerol esters (1) were started as far back as 1909, but the utility of the products as surface-active agents was not realized until sulfated monoglycerides of coconut oil fatty acids ($R\text{-}COO\text{-}CH_2CHOHCH_2OSO_3Na$) were put in the market under the trade name, Arctic Syntex-M,L, etc. However very little published information has appeared on the fundamental relations between constitution and properties. The present investigation on the comparative surface-active properties of the sodium salts of monoglyceride sulfates of C_{12}-, C_{14}-, C_{16}-, and C_{18}- saturated, oleic, and linoleic acids was undertaken with a view to elucidating any possible influence of the molecular weight and unsaturation of the fatty acids on the surface-active properties of the products of sulfation of the monoglycerides.

Raw Materials and Reagents Used

Fatty Acids. Lauric and myristic acids were separately crystallized from 25% solutions of the commercial products in acetone at −25°C. and −20°C., respectively. Four such crystallizations yielded the following products: lauric acid having neutralization equivalent (N.E.) 201.0, iodine value (I.V.) less than 0.1, m.p. 44.1°C., literature value 44.2°C., and myristic acid having N.E. 228.1, I.V. less than 0.1, m.p. 54.2°C., literature value 54.4°C. A 20% solution of palmitic acid in acetone was cooled to 5°C. to remove insoluble stearic acid. The filtrate was chilled to −12°C. and kept at that temperature for 2 hrs. Repeated crystallization gave palmitic acid having N. E. 257.2, I.V. nil, m.p. 62.7°C., literature value 62.9°C.

Three successive crystallizations of stearic acid from 10% solution in acetone at −5°C. for 3 hrs. gave a sufficiently pure stearic acid having N.E. 284.2, I.V. nil, m.p. 69.5°C., literature value 69.6°C.

Successive crystallizations of 7.5% of oleic acid solution in acetone at −25°C. and −35°C. precipitated crops of palmitic and oleic acid. Three recrystallizations of the oleic acid from acetone yielded a product

TABLE I

Analytical Characteristics of Monoglyceride Samples

	Saponification equivalent		Iodine value		Melting point		α-Monoglyceride
	Observed	Theoretical	Observed	Theoretical	Observed	Literature value	
					°C.	°C.	%
Monolaurin	273.4	274.0	Nil	Nil	61.9	62[4,5]	98.5
Monomyristin	299.8	302.0	Nil	Nil	69.9	70.5[6]	97.6
Monopalmitin	326.0	330.0	Nil	Nil	74.8	75[4]	97.5
Monostearin	354.6	358.0	Nil	Nil	80.2	80[4]	96.6
Mono-olein	354.8	356.0	71.2	72.1	......	......	98.6
Monolinolein	352.8	354.0	142.9	143.4	......	......	97.5

having N.E. 281.3, I.V. 90.81, and a linoleic acid content of 0.13%, as shown from the alkali isomerization and spectrophotometric determination (2). ($E^{1\%/1\text{cm.}}$ at 234 mμ of 180°C./60 min. alkali-isomerized sample was 1.18).

Linoleic acid was recovered from poppyseed oil m.f.a. Palmitic, stearic, and oleic acids were successively removed by chilling the acetone solution gradually to −35°C. Evaporation of the filtrate obtained after final cooling to −45°C. gave linoleic acid having N.E. 280.3, I.V 180.9, and a linoleic acid content of 98.2%. ($E^{1\%/1\text{cm.}}$ at 268 mμ and 234 mμ of 170°C./15 min. and 180°C./60 min. alkali-isomerized samples were, respectively, 5.56 and 895.7.)

Monoglycerides. The purified samples of fatty acids were then esterified with 3 equivalents of glycerol at 180°C. in a nitrogen atmosphere. The reaction was catalyzed with 0.1% potassium carbonate (on the basis of fat). After 5 hrs. the products were taken out, dissolved in ethyl ether, and freed from glycerol, fatty acid, and soap. The crude monoglyceride was then further purified by countercurrent distribution between two mutually saturated phases, alcohol-water and petroleum ether (b.p. 60–80°C.). Repeated crystallizations from ethyl ether of monolaurin and monomyristin at 5°C. and of monopalmitin and monostearin at 10°C. gave purer products.

The analytical figures for the purified monoglycerides, α-monoglyceride contents of which have been estimated according to the standard method (3), are reported in Table I.

Sulfating Reagents

There are references (7, 8) for preparing monoglyceride sulfates by reacting equimolar proportions of fatty acid, glycerol, and sulfuric acid. Products of low salt content have been made by using such reagents as sodium chlorosulfonate (9). Although sulfuric acid is a strong sulfating reagent, it attacks the double bond and partially splits the glyceride linkage; sodium chlorosulfonate is known to be a milder sulfating reagent. Recently a pyridine-sulfur trioxide complex has been used (10) for sulfation of unsaturated fatty alcohols and is shown to leave the double bonds virtually unaffected. In view of the above facts, a sodium chlorosulfonate and pyridine-sulfur trioxide complex have been used for sulfating monoglycerides of saturated and unsaturated acids, respectively. The pyridine-sulfur trioxide complex was prepared according to the directions given in H. H. Sisler and L. F. Audrieth's "Inorganic Synthesis" (Vol. II, p. 173, 1st ed., McGraw-Hill Book Company Inc., New York, 1946).

Experimental

Sulfation. Chlorosulfonic acid (0.26 mole) was added slowly with continuous stirring to a cold solution of sodium chloride (0.5 mole) until a white paste was obtained. This paste was gradually added to the molten monoglyceride (0.1 mole), and stirring was continued for 30 min. The mass was then dissolved in hot *n*-butanol and neutralized with 18 *n*-sodium hydroxide. The mixture was subsequently filtered hot to remove the insoluble inorganic salts. *n*-Butanol and water were finally removed by distillation under vacuum. The syrupy residue was poured into dry acetone, and the sodium salt of the sulfated monoglyceride was precipitated out. This was filtered, washed with dry acetone, and dried in vacuum.

In the case of an unsaturated fatty acid monoglyceride, 4.0 moles of a pyridine-sulfur trioxide complex were gradually added to 1.7 moles of monoglyceride with stirring. The temperature of the reaction mixture was maintained below 10°C. for 30 min., after which the mass was dissolved in hot *n*-butanol and neutralized with a hot sodium hydroxide solution. The subsequent procedure for obtaining the sodium salt of sulfated monoglycerides was similar to that outlined above.

Analysis of the Products

Percentages of sulfate and sulfonate groups (expressed as % of SO_3) in the products were estimated according to the A.O.C.S. Official Methods (F 2a-44 and F 2c-44). Content of sulfonate was negligible (less than 0.01%) in all the cases. Sulfate contents (expressed as % of SO_3) in the sodium salts of sulfated lauric, myristic, palmitic, stearic, oleic, and linoleic monoglycerides were found to be 21.01, 19.61, 18.32, 17.02, 17.19, and 17.27, respectively. (Corresponding theoretical values for monosulfated products are 21.27, 19.80, 18.52, 17.39, 17.47, and 17.54, respectively.) The iodine values of the products from mono-olein and monolinolein were 54.6 and 109.2, respectively (theoretical values 55.43 and 111.4, respectively). The following properties of the products have been studied.

a) *Surface Tension.* Surface tensions of 0.001 to 0.1% solutions of the surfactants were measured with a du Noüy's tensiometer at 25 ± 0.2°C. Values are given in Table II.

TABLE II

Surface Tension of Solutions of Sodium Salts of Fatty Acid Monoglyceride Sulfates (dynes/cm.). Temperature 25 ± 0.2°C.

	Fatty acid in the surfactant					
Concentration (%)	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic
0.001	71.0	52.2	70.6	71.9	64.2	64.3
0.002	69.0	46.0	67.1	71.9	61.2	61.1
0.005	56.1	38.5	55.3	59.1	57.7	58.2
0.01	50.5	37.8	49.1	49.2	55.7	56.0
0.02	42.0	33.6	47.5	48.1	49.5	49.5
0.05	36.3	27.2	46.1	46.0	43.1	43.2
0.1	33.9	26.1	45.9	46.0	36.0	37.1

b) *Interfacial Tension.* Determination of the interfacial tensions of the aqueous solutions of the surfactants against *n*-heptane (b.p. 208°F.) was done in du Noüy's tensiometer at 25 ± 0.2°C. The interfacial tension value of distilled water against *n*-heptane was 53.8 dynes/cm. Values obtained are presented in Table III.

TABLE III

Interfacial Tension of Surfactant Solutions (dynes/cm.). Temperature 25 ± 0.2°C.

	Fatty acid in the surfactant					
Concentration (%)	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic
0.005	53.8	51.1	52.0	53.8	53.8	50.2
0.01	52.0	46.8	47.3	52.2	51.8	46.1
0.02	48.5	39.4	42.6	46.5	43.6	40.0
0.05	44.7	29.9	32.7	35.3	32.7	32.7
0.10	38.9	18.8	21.4	25.6	21.7	25.2

c) *Wetting Characteristics.* Sinking-time tests were carried out according to the following procedure. A piece of cloth 2 by 2 in., rolled into a cylindrical shape and fastened by a 2-in. piece of cotton thread served as the experimental "cotton ball." The cotton ball was hooked by a curved 2-in. piece of copper wire, which was fastened to the end of a flattened glass rod by cotton thread (Figure 1). The whole assembly was immersed in the surfactant solution, maintained at a temperature of 25 ± 0.2°C. The time required for the cotton ball to sink was noted and was regarded as an inverse measure of the wetting efficiency of the surfactant solution. The sinking times in 0.1% solutions of sodium salts of monolaurin, -myristin, -palmitin, -stearin, -olein, and -linolein sulfates were 30 min., 87 min., >4 hrs., >4 hrs., 75 min., and 45 min., respectively. The sinking times in 0.025, 0.05, 0.1, and 0.2% solutions of the sodium salt of monolaurin sulfate were 139 min., 60 min., 30 min., and 19 min., respectively, which showed increased wetting efficiency with the increased concentration of surfactant.

d) *Calcium Stability.* Calcium stability was determined at 25 ± 1°C. according to the method of Harts (11). Fifty cc. of 0.05% solution of the surfactant in a 100-cc. beaker (5 cm. in internal diameter) were titrated with a 1% solution of calcium acetate until turbidity prevented ordinary newsprint, fastened to the side of the beaker with a rubber band, from being read when viewed through the solution. The burette readings (direct measures of calcium stability) in the case of the lauric, myristic, oleic, and linoleic acid products were 0.88, 0.55, 1.5, and 6.3 cc., respectively. (Calcium stability values expressed as p.p.m. $CaCO_3$ are therefore 109.5, 68.9, 184.3, and 708.2, respectively.) Minute quantities, less than 0.03 cc. of the same calcium acetate solution, made 0.05% solutions of the palmitic and stearic acid products turbid and produced coarse precipitates of calcium salts, thereby indicating calcium stability values of less than 4 in terms of p.p.m. $CaCO_3$.

e) *Foaming Characteristics.* Foam height and foam stability of the aqueous solutions of the surfactants were evaluated at 25 ± 1°C. on the principles underlying Ross and Miles' method (12). The 0.1% aqueous solution (200 cc.) was delivered from a pipette (*i.e.*, of a 1-mm. discharging nozzle) down a tall calibrated cylinder (45 mm. inner diameter) onto a cushion of 50 cc. of the same solution; the average height of fall was 72.5 cm. Foam heights and the half-foam life period, *i.e.*, the time required for the foam volume to be reduced to half the original value, are recorded in Table IV.

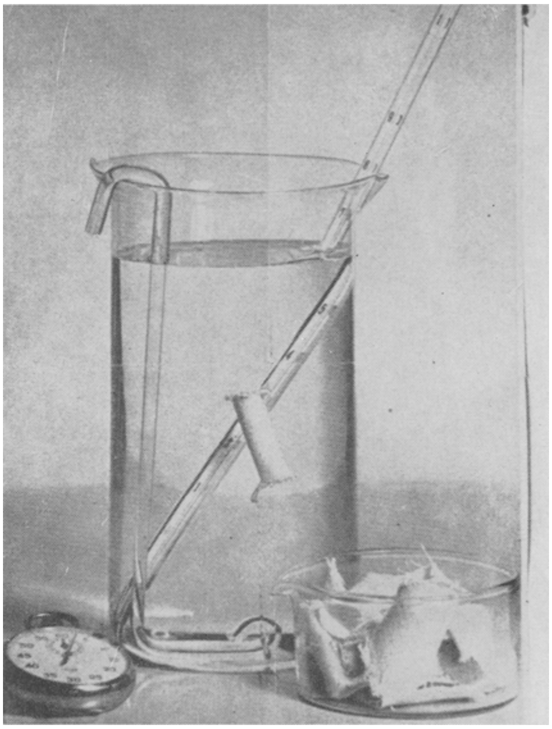

FIG. 1. Sinking time test assembly.

TABLE IV

Foam Height and Half-Life Period. Temperature 25 ± 1°C.

	Fatty acid in the surfactants (0.1% solutions)					
	Lauric	Myristic	Palmitic	Stearic	Oleic	Linoleic
Foam height in cm.	0.7	19.0	2.0	2.1	18.0	0.9
Half-life period (of the original foam volume)	1 min.	>1 hr.	45 min.	15 min.	>1 hr.	1 min.

f) *Suspendability Characteristic.* The suspendability of manganese dioxide particles in surfactant solutions at 25 ± 1°C. was evaluated by means of Fischer-Dott's apparatus, the working principle of which has been elaborated by Dotts (13). The apparatus consists of a vertically adjusted sedimentation tube attached to a calibrated capillary manometer arm and fitted with a stopcock. (The sine of the angle between the manometer arm and the horizontal is 0.075.) The dispersion contains 1 g. of MnO_2 (B. S. sieve size −70 to +100) in 100 cc. of 0.1% surfactant solution. With the manometer full of surfactant solution and the main sedimentation tube empty, a run is started by pouring the dispersion into the main tube to a predetermined mark, corresponding to which the meniscus reads zero in the manometer scale. The timer is started immediately, and the manometer stopcock is opened simultaneously. As the suspended solid particles settle, the effective density of the suspension on the top of the sedimentation tube falls, and the pressure on the manometer arm decreases with the result that the liquid meniscus in the manometer arm progressively recedes. The rate of recession serves as an inverse measure of the suspending capacity of the surfactant solution. The

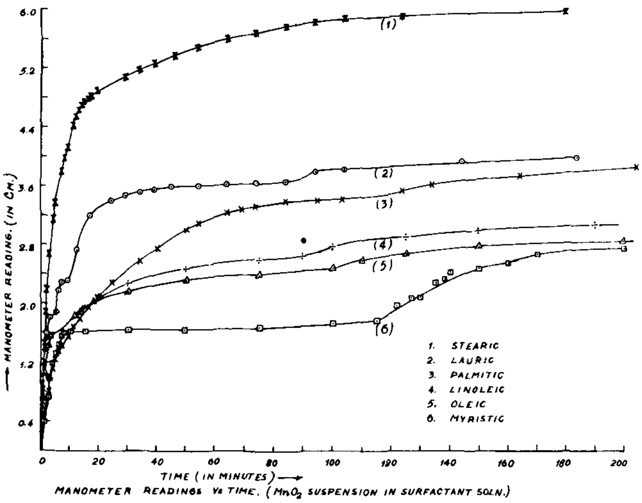

FIG. 2. Suspendability characteristics as determined in Fischer-Dott's apparatus.

results, as reproduced in Figure 2, indicate the superiority of the products in the following order:

Myristic → Oleic → Linoleic → Palmitic → Lauric → Stearic.

Thus, in the series C_{12}–C_{18} saturated compounds, maximum suspending power is exhibited by C_{14} compounds and, in the series of C_{18} compounds of increasing unsaturation, another maximum is attained by the oleic acid product.

g) *Emulsifying Property.* Fifteen cc. of 0.1% solution of the surfactant were shaken with 5 cc. of *n*-heptane (b. p. 208°F.) in a 25-cc. stoppered measuring cylinder (height 13 cm., inner diameter 1.75 cm.) for 1 hr. in a shaker, which imparted, on the average, 110 longitudinal shakings per minute; amplitude of vibration was 11 cm. Volumes of the emulsion layer, also of the aqueous and hydrocarbon layers, were noted at different intervals after the completion of shaking period. Data obtained are presented in Table V.

TABLE V

Phase Volumes at Various Times After Shaking 0.1% Surfactant Solution with *n*-Heptane. Temperature 25 ± 1°C.

Fatty acid in surf-actant	Time (min.)				
	0	5	15	30	60
Lauric	Em. 1.2 Oil 3.6	Same as in 0 min.	Em. 1.1 Oil 3.7	Em. 1.1 Oil 3.7	Em. 1.1 Oil 3.7
Myristic	Complete emulsification	Faint separation of phases.	Em. 5.5	Em. 5.4	Em. 5.4
Palmitic	Only 2 layers, one oily, the other coarse dispersion of oil in water	Oil 3.3	Oil 3.4	Oil 3.6	Oil 4.2
Stearic	Only 2 layers, one oily, the other coarse dispersion of oil in water	Oil 3.6	Oil 4.4	Oil 4.6 Aq. 1.5 Rest coarse dispersion	Oil 4.7 Aq. 5.3 Rest coarse dispersion
Oleic	Em. 5.2	Em. 5.1	Em. 5.0	Em. 5.0	Em. 5.0
Linoleic	Em. 5.6	Em. 5.5	Em. 5.0	Em. 4.9	Em. 4.9

Em. is volume (cc.) of emulsion layer. Oil is volume of heptane layer. Remainder is aqueous layer unless otherwise indicated.

Determination of the particle size of the oil droplets in the emulsions, at different intervals, which alone could give a correct idea about the nature of emulsion and its stability, was done according to the method outlined by King and Mukherji (14). One cc. of the emulsion layer was withdrawn in a pipette and mixed with 9 cc. of water and 1 cc. of 10% gelatin solution. A drop was taken from the middle of the mixed sample and placed on the Haemicytometer slide (Spencer Buffalo U.S.A. 7926, Neubauer. 1/10-mm. deep). Size distribution of the oil droplets was recorded from the microscopic observation. Surface area and volume of the droplets were computed from their diameters. Total surface area divided by total volume of the droplets gave a statistical average of surface area per unit volume of the oil droplets in emulsion and hence of the state of dispersion. The experiment was repeated with emulsion samples taken at different intervals, results of which are presented in Table VI.

TABLE VI

Specific Interface[a] of *n*-Heptane Emulsified in 0.1% Surfactant Solutions at Different Intervals

Fatty acid in the surfactant	Time after emulsification (hrs.)	Surface area/unit volume (sq. cm./cc.)	Specific interface (sq. cm./gm.) (σ)	ln σ
Lauric	0.5	1915	2821	7.945
	1	1741	2565	7.851
Myristic	1	3999	5892	8.682
	2	3395	5001	8.519
	24	749	1104	7.008
Palmitic	0.5	1710	2519	7.832
	1	1703	2509	7.828
Stearic	0.5	1716	2528	7.837
	1	1692	2493	7.823
Oleic	0.5	4659	6864	8.835
	1.25	3741	5512	8.616
	2	3545	5223	8.563
	24	2794	4116	8.324
Linoleic	1.25	2830	4170	8.337
	3	2250	3315	8.106
	24	379	559	6.328

[a] Specific interface is area/volume × density of the oil. Density of *n*-heptane at 25°C. = 0.6787.

The data reveal that lauric, palmitic, and stearic surfactants have negligible emulsion stability; the emulsion layer practically disappears within a few hours. In the cases of myristic, oleic, and linoleic products, emulsions were of sufficient stability. The natural logarithms of the specific interfaces have been plotted against time in Figure 3; the results show

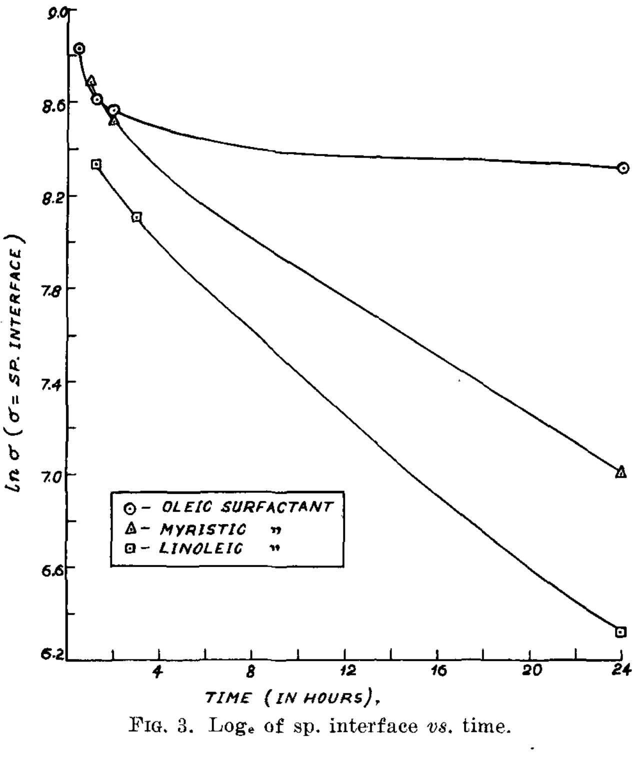

FIG. 3. Log_e of sp. interface *vs.* time.

that the superiority of the surfactants as an emulsifier is in the order, oleic>myristic>linoleic. According to Lotzkar and Maclay's (15) equation: $-\frac{d\sigma}{\sigma} = \frac{dt}{K}$ where K stands for the stability coefficient, the rate of change of specific interface, σ is exponential to time, t. Results obtained in the present work are at variance with Lotzkar's rule, as is shown by the fact that plottings $\ln\sigma$ *vs.* t are not straight lines but tend to be parallel to the abscissae. Thus the stability coefficient K is not constant but is found to increase with the passage of time. The increased emulsion stability most probably results from the hydrolysis product of the sodium salts, which, being more oil-compatible, stabilizes the emulsion. A combination of O/W and W/O type emulsifiers is known to be a good emulsion stabilizer.

Discussion

Surface tension and interfacial tension of aqueous solutions of this series of surfactants have been found to decrease with the increase in concentration. At equal concentrations the myristic surfactant (among the saturated acids) has been found to possess the best tensioactive properties of all the samples tested. The oleic product is the best of the three C_{18} surfactants of varying unsaturation. The same trend is also noted regarding other properties, *viz.*, foam characteristic, suspending and emulsifying capacity, and emulsion stability. Compared to the myristic surfactant, the oleic surfactant however gives a stabler emulsion at equal concentration.

The above trend is not strictly maintained with wetting characteristics. An increase in hydrophilic nature in the surfactant molecule appears to augment the property as evidenced in lauric and linoleic products. This may be attributed to the high rate of adsorption on and diffusion through the cotton ball. Calcium stability is also found to be better the more the surfactant is water-soluble, *e.g.*, linoleic, oleic, and lauric products.

It may be proposed as a general rule that an optimum balance between hydrophilic and hydrophobic groups in the surfactant molecule is contributory to the improvement in surface-active properties. The hydrophobic character of the fatty acids develops as their molecular weight increases while with the increase in unsaturation the molecule is rendered more hydrophilic.

The role of this optimum hydrophilic and hydrophobic balance on surface-active properties has been worked out by Weil *et al.* (10) and Kivalo (16), respectively, in sulfated fatty alcohols and sodium soaps. Weil *et al.* have established that, in the sodium salts of sulfated fatty alcohols, surface tension-lowering capacity of 0.1% solution is maximum in the case of the C_{14} surfactant and the foaming characteristic of 0.25% solution is maximum at C_{16}. Kivalo (16) has shown that at a 2-millimolar concentration sodium oleate has a better tensio-active property than either stearate or linoleate. From the present investigation it is evident that sulfated monoglycerides of myristic and oleic acids have the nearest approach to the state of optimum hydrophilic-hydrophobic balance.

Summary

Sodium salts of fatty acid monoglyceride sulfates have been prepared by sulfating purified monoglycerides with chlorosulfonic acid or a pyridine-sulfur trioxide complex and subsequent neutralizing. Surface tension, interfacial tension, wetting, suspending and foaming characteristics, calcium stability, and emulsifying property (including stability of the emulsion obtained) of the aqueous solution of these surfactants from C_{12}, C_{14}, C_{16}, and C_{18} saturated fatty acids and from oleic and linoleic acids have been determined and evaluated.

With the exception of the wetting characteristic which was found to be optimum in the case of the more hydrophilic products (like those from lauric and linoleic acids) the remaining properties were found to reach a maximum value when the hydrophilic-hydrophobic balance in the surfactant molecule was seemingly optimum, as attained by myristic and oleic acid products.

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