used. Corrections may also be necessary if the BHT/BHA ratio is high.

*Extraction and Recovery of BHA from Fats.* Solvent extraction was the preferred method for recovery of BHA. It permits the separation of BHA from BHT (if present) and allows a higher BHA concentration than other methods.

TABLE I
Recovery of BHA from Lard

| Lot and sample No. | Weight of sample g. | Antioxidant added to 200 g. lard, mg. | | BHA found in 100 g. lard, mg. |
|---|---|---|---|---|
| $A_1$ | 10 | 10.0 | BHA | 9.6 |
| $A_2$ | 10 | 10.0 | BHA | 9.6 |
| $A_3$ | 10 | 10.0 | BHA | 9.6 |
| $A_4$ | 10 | 10.0 | BHA | 9.8 |
| | | 3.0 | PG [a] | |
| $A_5$ | 10 | 10.0 | BHA | 9.8 |
| | | 3.0 | PG | |
| $B_1$ | 10 | 10.0 | BHA | 10.1 |
| $B_2$ | 10 | 10.0 | BHA | 10.1 |
| $B_3$ | 15 | 10.0 | BHA | 10.7 |
| $C_1$ | 10 | 5.0 | BHA | 4.6 |
| $C_2$ | 15 | 5.0 | BHA | 5.0 |
| $C_3$ | 15 | 5.0 | BHA | 5.0 |
| $D_1$ | 15 | 10.0 | BHA | 9.7 |
| | | 10.0 | BHT | |

[a] Propyl gallate.

Various difficulties involving turbidity, emulsion formation, or interference with maximum color development were encountered. These ruled out extraction with 72% ethanol from a solution of fat in cyclohexane (5), extraction with methanol from a chloroform solution of fat (1), or direct extraction with 72% ethanol stirred through fat with a Blendor.

Best results were obtained with the modification of the method of Mahon and Chapman (4), which involved extraction from a solution of a larger sample (15 g.) of lard dissolved in petroleum ether since this resulted in better recovery of BHA. When propyl gallate was present, it was removed prior to extraction of the BHA (3).

Freshly rendered lard with a peroxide value of 1.5 m.e./kg. was used for the recovery studies. The BHA was extracted immediately after incorporation because repeated warming and cooling of a fat containing BHA resulted in a significant lowering of BHA concentration in a very short time. Whether this is due to loss of antioxidant by volatilization or conversion to forms which no longer give the color reaction has not been determined.

*Determination of BHA in Fats and Oils.* Transfer 15 g. of melted fat or oil to a 500-ml. separatory funnel with the aid of 50 ml. of petroleum ether (b.p. 30–60°C.). Extract with three 25-ml. portions of 72% ethanol by continuously inverting the funnel for 3 min. Follow with a 1-min. extraction, using 60 ml. of 72% ethanol. Let the phases separate well between each extraction. Filter the combined extracts through 2 Whatman #54 filter papers and make up to a final volume of 150 ml. with 72% ethanol.

If propyl gallate is present, it should be extracted from the dissolved fat by the methods of Mahon and Chapman (3) prior to the extraction with ethanol.

Add 2 ml. of cold Ehrlich reagent (1:100) to 7 ml. of the extract and then, drop by drop, add 1 ml. of 7 N NaOH with constant shaking. Read the optical density after 10 min. in a spectrophotometer at 535 mμ, using a blank of 7 ml. 72% ethanol, 2 ml. of Ehrlich reagent, and 1 ml. of 7 N NaOH. (Blanks made of extracts of the same lard containing no BHA differ from the 72% ethanol blank only by the range of error of the method.)

Calculate the quantity of BHA in the fat in percentage from the following equation:

$$\% \text{ BHA in fat} = (1000/7)c$$

c = concentration of BHA in the 10-ml. final volume of the determination. Determine "c" from the standard curve.

*Precision of the Method.* The precision of the method has been checked by using different aliquots of a 72% ethanolic extract from fat containing BHA and making them up to 7 ml. with 72% ethanol prior to color development. The BHA concentration also was measured in 6 ml. of the extract plus 1 ml. of a solution of known concentration of BHA in 72% ethanol. The deviations ranged from 0.0001 to less than 0.0003% of the BHA in the fat or 1 to less than 3% of the antioxidant added (0.01%).

REFERENCES

1. Hansen, P.V., Kaufman, F.L., and Wiedermann, L.H., J. Am. Oil Chemists' Soc., *36*, 193–195 (1959).
2. Laszlo, H., Eng. e quim. (Rio de Janeiro), *9*, No. 5, 1–5 (1957).
3. Mahon, J.H., and Chapman, R.A., Anal. Chem., *23*, 1116–1120 (1951).
4. Mahon, J.H., and Chapman, R.A., Anal. Chem., *23*, 1120–1123 (1951).
5. Wolff, J.P., Rev. franc. corps gras, *5*, 630–40 (1958).

[Received July 2, 1959]

# Report of the Spectroscopy Committee, 1959-60

AT A MEETING held at the Roosevelt hotel, New Orleans, La., during the 50th Annual Meeting of the Society, April 20–22, 1959, the Spectroscopy Committee decided that collaborative tests to extend the scope of the infrared absorption method for isolated *trans* ethylenic bonds[1] to the analysis of long-chain fatty acids directly should be the next activity. It was also decided that efforts to make the secondary standards, required by users of this method, readily available should be expedited. Accordingly during the past year collaborative investigation of an analysis of fatty acids directly for their *trans* content, *i.e.*, without prior conversion to the methyl esters, has been undertaken. In addition, fatty acid methyl esters and triglycerides of high and low *trans* isomer content have been analyzed by the entire committee and established as secondary standards for the method as published (1).

A single meeting was held during the year, in connection with the Annual Meeting in Dallas, April 4–6, 1960.

[1] Hereinafter in this report referred to as "*trans* content" or "*trans*-isomers."

## Present Status

Following collaborative work a year ago, the committee recommended to the Uniform Methods Committee a proposed A.O.C.S. Tentative Method for Isolated *trans*-Isomers by Means of Infrared Absorption Spectrophotometry (1). This method provided techniques for the determination of *trans* isomers in natural or processed esters and triglycerides of long-chain fatty acids but required that the free long-chain fatty acids be converted to their methyl esters prior to analysis. The Uniform Methods Committee has objected to adoption of this method as an A.O.C.S. Tentative Method until a technique for the conversion of the fatty acid samples to their methyl esters could be included. Accordingly a section entitled "Preparation of Methyl Esters of Fatty Acids by Use of Diazomethane" was added to the method. However review of this proposed change made it quite evident that there is considerable disagreement among oil chemists as to how a long-chain fatty acid can most suitably be converted to its methyl ester. Attempts to conciliate various views have held up adoption of the Infrared Absorption Method as a Tentative A.O.C.S. method.

At the 1960 meeting of the Spectroscopy Committee this problem was considered, and after considerable discussion it was unanimously agreed by the seven of the 10-member committee present that incorporation into the Isolated *trans*-Isomers—Infrared Spectrophotometric Method of the experimental procedure from an unpublished modification of the diazomethane method entitled "The Esterification of Fatty Acids with Diazomethane on a Small Scale" by Hermann Schlenk and Joanne L. Gellerman, of the Hormel Institute, University of Minnesota, Austin, Minn., would be most satisfactory. J.R. Chipault, a member of the committee from the Hormel Institute, has provided copies of the procedure.

As a result of decisions at the 1960 meeting in Dallas the method, as printed in the Journal (1), will be modified not only to include this procedure for converting the long-chain fatty acids to their methyl esters, where required, but (see below) will include a provision for the analysis of *trans*-isomers in long-chain fatty acids directly where the *trans*-isomer content is above 15%. Provision for long-chain fatty acid secondary standards will be added to these already available for the esters and triglycerides of long-chain fatty acids. All of these secondary standards will continue to be available for distribution by the chairman of the Spectroscopy Committee.

## Collaborative Work

As a collaborative test to extend the scope of the infrared absorption method for isolated *trans* to the analysis of long-chain fatty acids directly, 11 samples were furnished each committee member as follows:

a) *samples to establish secondary standards:*

No. 1. Triglyceride-*trans* content *ca.* 50%
No. 2. Triglyceride-*trans* content *ca.* 20%
No. 3. Methyl Ester-*trans* content *ca.* 40%
No. 4. Methyl Ester-*trans* content *ca.* 10%

b) *samples to test direct analysis of fatty acids:*

Two primary standards
Elaidic acids *ca.* 99+%
Five analytical samples:
No. 1 through No. 5, *trans* content 3—40%

Each committee member was asked to analyze each sample at least in duplicate on at least two different days, on as many different infrared spectrophotometers as were available to him. Results were received from all 10 members of the Spectroscopy Committee and are given in Tables I and II. A statistical analysis of their data is given in Table III.

Considering the test materials (after dropping analytical sample No. 2) as entities for study, the data were analyzed in eight analyses, one for each material. Considering the 11 instruments (10 laboratories with two instruments at one) as entities, the same data were analyzed in 11 analyses, one for each instrument.

The eight standard deviations of determinations within days for the several samples did not seem to increase with the general level of percentage of *trans* in the samples as often happened with such data and as apparently happened last year.

The standard deviations of both days and determinations within days seemed to be considerably higher for one instrument than for the others. A test of significance revealed significant differences among the instruments in this respect so that the instrument with greater internal variability within samples was deleted from the statistical treatment of the study illustrated in Table III. The standard deviations as recomputed for eight of the test materials, omitting the questionable instrument, did show a tendency to increase as the percentage of *trans* in the samples increased. This ability to see an expected trend after freeing the remaining results of the obscuring effects of the highly variable data from the one instrument is regarded as further evidence that dropping the variable instrument is a justified procedure if the objective is to determine something of the variability of the procedure in laboratories which seem to be in control.

The results from the remaining 10 instruments were analyzed by the method developed by Mandel and his associates at the National Bureau of Standards (2, 3, 4). Since the standard deviations were found to be related to the sample means as follows:

$$(1) \quad \text{standard deviation} = 0.264 + (0.007)(\%\ trans),$$

the data were transformed as follows:

$$(2) \qquad Z = 1000\ (1 + \log\ (0.264 + 0.007y))$$

where Z is the transformation of y, and y is the average percentage of *trans* for two determinations on each of two days. The primary findings of the analysis of the transformed data are summarized in Table III.

Table III shows, for the arbitrarily selected values of y = 10, 20, 30, 40, and 50 percentage of *trans*, the transformation values, Z, of such *trans* percentages. Also shown are the estimated variances of single observations on materials of this *trans* content expressed in several ways: $\hat{V}(Z)$ = estimated variance of transformed units, $\hat{V}(y)$ = estimated variance of natural units, $\sqrt{\hat{V}(y)}$ = standard deviation of natural units, and C.V. = coefficient of variation = standard deviation expressed as a percentage of the mean percentage of *trans*. One may note that the variance of transformed units, $\hat{V}(Z)$, does not seem to be related to the average *trans* value, though both the variance, $\hat{V}(y)$, and standard deviation, $\sqrt{\hat{V}(y)}$, of natural units do increase with the increased percentage of *trans*. Though materials of higher *trans* content have greater absolute variations ($\sqrt{\hat{V}(y)}$ in Table III) than materials of lower content, these variations are smaller by per-

TABLE I

Spectroscopy Committee Collaborative Testing for Isolated *Trans*

| Collaborator No. | Date and instrument | Analytical Samples | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Long-chain fatty acid No. 1 | | | Long-chain fatty acid No. 2 | | | Long-chain fatty acid No. 3 | | | Long-chain fatty acid No. 4 | | | Long-chain fatty acid No. 5 | | |
| | | a | % Trans | Deviation from mean | a | % Trans | Deviation from mean | a | % Trans | Deviation from mean | a | % Trans | Deviation from mean | a | % Trans | Deviation from mean |
| 1 | 1st day | 0.182 | 40.3 | 1.47 | 0.014 | 3.1 | 0.31 | 0.035 | 7.8 | 0.42 | 0.069 | 15.3 | 0.70 | 0.122 | 27.1 | 1.78 |
| | 2nd day Perkin-Elmer 21 | 0.177 | 39.2 | 0.37 | 0.015 | 3.4 | 0.01 | 0.032 | 7.1 | 0.28 | 0.069 | 15.4 | 0.80 | 0.118 | 26.3 | 0.98 |
| 2 | 1st day | 0.172 | 38.0 | 0.83 | 0.020 | 4.4 | 0.99 | 0.038 | 8.6 | 1.22 | 0.068 | 15.1 | 0.50 | 0.114 | 25.4 | 0.08 |
| | 2nd day Perkin-Elmer 21 | 0.170 | 37.8 | 1.03 | 0.020 | 4.6 | 1.19 | 0.038 | 8.6 | 1.22 | 0.067 | 14.8 | 0.20 | 0.110 | 24.2 | 1.12 |
| 3 | 1st day | 0.192 | 38.5 | 0.33 | | | | 0.039 | 7.8 | 0.42 | 0.066 | 13.3 | 1.30 | 0.124 | 24.8 | 0.52 |
| | 2nd day Perkin-Elmer 21 | 0.194 | 38.8 | 0.03 | | | | 0.037 | 7.4 | 0.02 | 0.069 | 13.8 | 0.80 | 0.126 | 25.2 | 0.12 |
| 4 | 1st day | 0.189 | 38.6 | 0.23 | | | | 0.042 | 8.7 | 1.32 | 0.075 | 15.3 | 0.70 | 0.126 | 25.7 | 0.38 |
| | 2nd day IR-4 | 0.206 | 40.6 | 1.77 | | | | 0.044 | 8.7 | 1.32 | 0.079 | 15.6 | 1.00 | 0.135 | 26.6 | 1.28 |
| 5 | 1st day | 0.184 | 39.0 | 0.17 | 0.022 | 4.6 | 1.19 | 0.037 | 8.0 | 0.62 | 0.068 | 14.6 | 0.00 | 0.110 | 23.7 | 1.62 |
| | 2nd day Perkin-Elmer 21 | 0.175 | 38.4 | 0.43 | 0.015 | 3.3 | 0.11 | 0.035 | 7.7 | 0.32 | 0.068 | 14.9 | 0.30 | 0.109 | 24.0 | 1.32 |
| 6 | 1st day | 0.172 | 37.0 | 1.83 | 0.007 | 1.5 | 1.91 | 0.029 | 6.2 | 1.18 | 0.062 | 13.3 | 1.30 | 0.114 | 24.6 | 0.72 |
| | 2nd day IR-4 | 0.170 | 36.7 | 2.13 | 0.008 | 1.6 | 1.81 | 0.030 | 6.4 | 0.98 | 0.064 | 13.8 | 0.80 | 0.112 | 24.2 | 1.12 |
| 6 | 1st day | 0.182 | 38.8 | 0.03 | 0.012 | 2.5 | 0.91 | 0.030 | 6.0 | 1.38 | 0.069 | 14.8 | 0.20 | 0.123 | 26.2 | 0.88 |
| | 2nd day IR-5 | 0.183 | 39.1 | 0.27 | 0.011 | 2.4 | 1.01 | 0.030 | 6.5 | 0.88 | 0.068 | 14.6 | 0.00 | 0.120 | 25.8 | 0.48 |
| 7 | 1st day | 0.182 | 39.0 | 0.17 | 0.031 | 6.6 | 3.19 | 0.040 | 8.6 | 1.22 | 0.072 | 15.3 | 0.70 | 0.120 | 25.8 | 0.48 |
| | 2nd day Perkin-Elmer 21 | 0.182 | 39.0 | 0.17 | 0.027 | 5.8 | 2.39 | 0.039 | 8.4 | 1.02 | 0.072 | 15.3 | 0.70 | 0.118 | 25.3 | 0.02 |
| 8 | 1st day | 0.174 | 38.2 | 0.63 | 0.008 | 1.6 | 1.81 | 0.027 | 6.0 | 1.38 | 0.062 | 13.6 | 1.00 | 0.112 | 24.6 | 0.72 |
| | 2nd day IR-4 | 0.180 | 38.4 | 0.43 | 0.008 | 1.8 | 1.61 | 0.032 | 6.9 | 0.48 | 0.070 | 15.0 | 0.40 | 0.118 | 25.3 | 0.02 |
| 9 | 1st day | 0.180 | 38.8 | 0.03 | 0.016 | 3.6 | 0.19 | 0.038 | 8.3 | 0.92 | 0.073 | 15.7 | 1.10 | 0.122 | 26.4 | 1.08 |
| | 2nd day Perkin-Elmer 21 | 0.178 | 38.4 | 0.43 | 0.018 | 3.8 | 0.39 | 0.040 | 8.5 | 1.12 | 0.073 | 15.7 | 1.10 | 0.118 | 25.5 | 0.18 |
| 10 | 1st day | 0.184 | 41.7 | 2.87 | | | | 0.022 | 5.1 | 2.28 | 0.057 | 12.9 | 1.70 | 0.111 | 25.2 | 0.12 |
| | 2nd day Perkin-Elmer 21 | 0.176 | 39.9 | 1.07 | | | | 0.022 | 5.0 | 2.38 | 0.057 | 13.0 | 1.60 | 0.111 | 25.2 | 0.12 |
| | Average | | 38.83 | 0.76 | | 3.41 | 1.19 | | 7.38 | 1.02 | | 14.60 | 0.77 | | 25.32 | 0.69 |
| | Standard deviation | | | 1.11 | | | 1.52 | | | 1.20 | | | 0.92 | | | 0.89 |

TABLE II

Spectroscopy Committee Collaborative Testing for Isolated *Trans*

| Collaborator No. | Date and instrument | Primary Standards | | Secondary Standards | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Elaidic acid No. 1 | Elaidic acid No. 2 | Triglyceride No. 1 | | | Triglyceride No. 2 | | | Methyl ester No. 3 | | | Methyl ester No. 4 | | |
| | | a | a | a | % Trans | Deviation from mean | a | % Trans | Deviation from mean | a | % Trans | Deviation from mean | a | % Trans | Deviation from mean |
| 1 | 1st day | 0.452 | 0.453 | 0.233 | 50.5 | 1.39 | 0.110 | 23.9 | 0.17 | 0.175 | 37.3 | 0.85 | 0.045 | 9.5 | 0.66 |
| | 2nd day Perkin-Elmer 21 | 0.447 | 0.453 | 0.230 | 49.8 | 2.09 | 0.109 | 23.7 | 0.37 | 0.178 | 38.0 | 0.15 | 0.046 | 9.9 | 0.26 |
| 2 | 1st day | 0.448 | 0.453 | 0.224 | 50.6 | 1.29 | 0.104 | 23.6 | 0.47 | 0.164 | 36.2 | 1.95 | 0.042 | 9.3 | 0.86 |
| | 2nd day Perkin-Elmer 21 | 0.452 | 0.451 | 0.220 | 50.0 | 1.89 | 0.102 | 23.2 | 0.87 | 0.164 | 36.3 | 1.85 | 0.042 | 9.4 | 0.76 |
| 3 | 1st day | 0.490 | 0.506 | 0.248 | 51.4 | 0.49 | 0.118 | 24.4 | 0.33 | 0.182 | 37.2 | 0.95 | 0.053 | 10.8 | 0.64 |
| | 2nd day Perkin-Elmer 21 | 0.494 | 0.505 | 0.248 | 51.4 | 0.49 | 0.118 | 24.4 | 0.33 | 0.184 | 37.8 | 0.35 | 0.054 | 11.0 | 0.84 |
| 4 | 1st day | 0.489 | 0.502 | 0.246 | 50.4 | 1.49 | 0.111 | 22.8 | 1.27 | 0.178 | 36.2 | 1.95 | 0.050 | 10.2 | 0.04 |
| | 2nd day IR-4 | 0.490 | 0.511 | 0.255 | 50.4 | 1.49 | 0.115 | 22.6 | 1.47 | 0.189 | 37.4 | 0.75 | 0.053 | 10.1 | 0.06 |
| 5 | 1st day | 0.458 | 0.465 | 0.231 | 51.7 | 0.19 | 0.110 | 24.7 | 0.63 | 0.178 | 40.5 | 2.35 | 0.046 | 10.5 | 0.34 |
| | 2nd day Perkin-Elmer 21 | 0.448 | 0.454 | 0.234 | 53.3 | 1.41 | 0.108 | 24.5 | 0.43 | 0.175 | 39.8 | 1.65 | 0.046 | 10.6 | 0.44 |
| 6 | 1st day | 0.464 | 0.467 | 0.234 | 54.8 | 2.91 | 0.106 | 24.9 | 0.83 | 0.172 | 39.5 | 1.35 | 0.047 | 10.8 | 0.64 |
| | 2nd day IR-4 | 0.458 | 0.466 | 0.233 | 54.6 | 2.71 | 0.106 | 24.9 | 0.83 | 0.172 | 39.4 | 1.25 | 0.047 | 10.7 | 0.54 |
| 6 | 1st day | 0.454 | 0.468 | 0.235 | 53.6 | 1.71 | 0.108 | 24.6 | 0.53 | 0.172 | 38.4 | 0.25 | 0.048 | 10.1 | 0.06 |
| | 2nd day IR-5 | 0.456 | 0.467 | 0.234 | 53.3 | 1.41 | 0.106 | 24.3 | 0.23 | 0.172 | 38.5 | 0.35 | 0.044 | 10.0 | 0.16 |
| 7 | 1st day | 0.454 | 0.470 | 0.240 | 52.6 | 0.71 | 0.114 | 25.2 | 1.13 | 0.178 | 38.6 | 0.45 | 0.040 | 8.7 | 1.46 |
| | 2nd day Perkin-Elmer 21 | 0.462 | 0.466 | 0.238 | 52.4 | 0.51 | 0.120 | 26.2 | 2.13 | 0.176 | 38.3 | 0.15 | 0.047 | 10.2 | 0.04 |
| 8 | 1st day | 0.456 | 0.452 | 0.236 | 54.0 | 2.11 | 0.110 | 25.0 | 0.93 | 0.174 | 38.2 | 0.05 | 0.048 | 10.5 | 0.34 |
| | 2nd day IR-4 | 0.466 | 0.468 | 0.235 | 53.9 | 2.01 | 0.112 | 25.6 | 1.53 | 0.178 | 39.0 | 0.85 | 0.046 | 10.1 | 0.06 |
| 9 | 1st day | 0.456 | 0.461 | 0.228 | 50.9 | 0.99 | 0.105 | 22.2 | 1.87 | 0.166 | 38.4 | 0.25 | 0.042 | 11.4 | 1.24 |
| | 2nd day Perkin-Elmer 21 | 0.456 | 0.468 | 0.230 | 51.4 | 0.49 | 0.108 | 22.8 | 1.27 | 0.169 | 39.2 | 1.05 | 0.042 | 11.4 | 1.24 |
| 10 | 1st day | 0.433 | 0.436 | 0.239 | 50.2 | 1.69 | 0.109 | 23.0 | 1.07 | 0.180 | 37.7 | 0.45 | 0.045 | 9.3 | 0.86 |
| | 2nd day Perkin-Elmer 21 | 0.446 | 0.451 | 0.240 | 50.3 | 1.59 | 0.109 | 23.0 | 1.07 | 0.179 | 37.4 | 0.75 | 0.044 | 9.1 | 1.06 |
| | Average | | | | 51.89 | 1.41 | | 24.07 | 0.90 | | 38.15 | 0.91 | | 10.16 | 0.57 |
| | Standard Deviation | | | | | 1.62 | | | 1.07 | | | 1.16 | | | 0.73 |

TABLE III

Estimated Total Variance of Individual Determinations, and Proportion of Variance Attributable to Several Sources, for Samples at Several Selected Levels of Percentage of *trans*

| Item of information | Selected levels = aver. *trans* value | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Level of percentage of *trans* in: | | | | | |
| 1. Natural units, y = % *trans* | 10 | 20 | 30 | 40 | 50 |
| 2. Transformed units, Z[a] | 524 | 606 | 676 | 736 | 788 |
| Variance of a single observation: | | | | | |
| 1. $\hat{V}(Z)$[b] | 73.96 | 57.71 | 55.31 | 61.68 | 73.43 |
| 2. $\hat{V}(y)$[c] | 0.89 | 1.02 | 1.34 | 1.97 | 2.99 |
| 3. Standard deviation = $\sqrt{\hat{V}(y)}$ | 0.94 | 1.01 | 1.16 | 1.40 | 1.73 |
| 4. Standard deviation as % of mean, C.V. | 9.4 | 5.0 | 3.9 | 3.5 | 3.5 |
| Percentage of variance attributable to: | | | | | |
| 1. $\hat{V}(\epsilon)$[d] [= $\hat{V}(\epsilon)$] | 12.5 | 16.0 | 16.7 | 15.0 | 12.6 |
| 2. $\hat{V}(\lambda)$[e] [= $\hat{V}(\lambda)$] | 58.5 | 74.9 | 78.1 | 70.0 | 58.9 |
| 3. $\hat{V}(\mu)$[f] [= $[1 + \alpha(\bar{Z} - \bar{\bar{Z}})]^2\hat{V}(\mu)$] | 11.7 | 7.2 | 2.9 | 0.6 | 0.0 |
| 4. $\hat{V}(\delta)$[g] [= $(\bar{Z} - \bar{\bar{Z}})^2\hat{V}(\delta)$] | 17.4 | 1.9 | 2.2 | 14.3 | 28.6 |

[a] $Z = 1000[1 + \log (0.264 + 0.007y)]$

[b] $\hat{V}(Z) = \hat{V}(\epsilon) + \hat{V}(\lambda) + [1 + \alpha(\bar{Z} - \bar{\bar{Z}})]^2\hat{V}(\mu) + (\bar{Z} - \bar{\bar{Z}})^2\hat{V}(\delta)$

[c] $\hat{V}(y) = \left[\frac{2.3}{1000}\left(\frac{0.264}{0.007} + \text{ave } y\right)\right]^2\hat{V}(\bar{Z})$

[d] $\hat{V}(\epsilon) = \left[\frac{(1000)(0.007)}{2.3}\right]^2$. This is the variability among replicate determinations. It can be reduced to any specified level by sufficient replications.

[e] $\hat{V}(\lambda) = \hat{V}(\eta) - \frac{\hat{V}(\epsilon)}{\text{No. of replicates}}$ where $\hat{V}(\eta)$ = the average over all laboratories of $\left(\frac{\text{no. of labs.}}{\text{no. of labs.} - 1}\right)$ × (mean square of deviations from the linear regression). $\hat{V}(\lambda)$ is the failure of the average values found by a laboratory to fit exactly the best line of relationship (linear) between this laboratory's results and the average results of all laboratories after allowing for $\hat{V}(\epsilon)$. It represents, among other things, the differential response of different laboratories to the various interfering properties and disturbing conditions. $\hat{V}(\lambda)$ has been called the irreducible within-laboratory error; however it is only irreducible by replication. It can be reduced by further refinement of the procedure.

[f] $\hat{V}(\mu) = \frac{\text{Lab. MS} - \hat{V}(\eta)}{\text{no. of samples}}$ where $\hat{V}(\eta)$ is as described in [e] $[1 + \alpha(\bar{Z} - \bar{\bar{Z}})]^2\hat{V}(\mu)$ measures general differences among laboratories for samples at a particular mean level, taking into account,

1. any tendency for concurrence among the individual laboratory responses,
2. the relationship, $\alpha$, between laboratory mean, $\mu$, and the slope, $\beta$, of its response line, and
3. the direction and distance of the particular level from the concurrency (determined automatically by $\alpha$ and the mean value under consideration).

[g] $\hat{V}(\delta) = \hat{V}(\beta) - \alpha^2\hat{V}(\mu)$ where $\hat{V}(\beta) = (\text{no. of labs.})\frac{\text{Lab.} \times \text{Mat. MS} - \hat{V}(\eta)}{\text{Mat. MS} - \hat{V}(\eta)}$

where $\hat{V}(\eta)$ is as in [e]. This represents differences among laboratories because of differences among the slopes of the lines of response not explained by or related to $\mu$, the average level. If $\hat{V}(\delta)$ does not exist or is negligibly small, the lines will converge, be concurrent, at some point, or perhaps be parallel. Then a laboratory may be calibrated by using a standard at some distance from the point of concurrence. If $\hat{V}(\delta)$ is sizable, calibration must be made at two points.

centage of C.V. than the variations in samples of lower *trans* content.

Table III reports the proportion of the total variance of an observation, either $\hat{V}(Z)$ or $\hat{V}(y)$, attributable to each of four scources:

a) $\hat{V}(\epsilon)$ representing the variability among replicate determinations within a laboratory,

b) $\hat{V}(\lambda)$ representing the variability of individual test materials from the straight line best relating the results of a particular laboratory to the average of all laboratories (or due to individual laboratory peculiarities with particular materials),

c) $[1 + \alpha(\bar{Z} - \bar{\bar{Z}})]^2\hat{V}(\mu)$ representing the variability along laboratories weighted by tendency to concurrence and distance of the point under consideration from the region of concurrence, and

d) $(\bar{Z} - \bar{\bar{Z}})^2\hat{V}(\delta)$ representing the random criss-crossing of the lines of response weighted by the distance of the mean under consideration from the mean of all means.

It is apparent from Table II that the major portion of the uncertainty or variance of a determination is due to $\hat{V}(\lambda)$, the so-called "irreducible" within laboratory variance. Noting that this irreducibility refers to the fact that this component cannot be reduced by further replication within laboratories, though it can be reduced by further modifying the procedure to reduce or eliminate the causes of different results by different laboratories, it would seem that the next step in the development of this particular procedure might be a case study of those laboratories contributing most to $\hat{V}(\lambda)$ to determine, if possible, why their results should be so discrepant from the other laboratories.

## Committee Meeting in Dallas

The committee met in the Baker hotel on April 5, 1960. Seven of the 10 committee members were present, and two guests attended:

Members

Robert R. Allen, Anderson Clayton and Company
J. R. Chipault, Hormel Institute, University of Minnesota
Ralph E. Kelley, Hercules Powder Company
William E. Link, Archer-Daniels-Midland Company
Donald H. Wheeler, General Mills Inc.
Hans Wolff, A. E. Staley Manufacturing Company
Robert T. O'Connor, Southern Utilization Research and Development Division, U.S.D.A.

Guests

Russell Walker, Anderson Clayton and Company
R. C. Stillman, Procter and Gamble

*Discussion of Results of Collaborative Tests.* The recently completed collaborative work designed to test the advisability of extending the proposed method for isolated *trans*-isomers by infrared absorption to the analysis of long-chain fatty acids directly was discussed in detail, and the following conclusions were unanimously agreed upon:

a) The results of the collaborative work to determine isolated *trans* in long-chain fatty acid directly show that satisfactory values can be obtained if the *trans* content is above a minimum value. However, if the *trans* content is too low, the excessive background correction, arising from the proximity of the strong COOH absorption band, makes the results of the analysis unreliable. (See attached tables of collaborative results.) After discussion it was agreed that the lower limit for which isolated *trans* should be determined by analysis of the long-chain fatty acid directly is 15%. If the *trans* content is below 15%, the acid should be converted to its methyl ester for satisfactory analysis.

b) It was agreed that the proposed method should be modified to permit the direct analysis of long-chain fatty acids for isolated *trans* content in all samples where this value is 15% or greater and that secondary standards should be prepared for distribution along with the secondary standards for esters and triglycerides.

c) For fatty acids with an isolated *trans* content below 15%, the proposed method should describe a suitable procedure for conversion to the methyl esters and the analyses should be made as provided for analysis of esters.

*Discussion of Uniform Methods Request for Description of Technique for Conversion of Long-Chain Fatty Acid Samples (Where Required) to Their Methyl Esters.* A poll of the nine members of the Spectroscopy Committee (not including the chairman) showed that two favored the diazomethane method for converting long-chain fatty acids to their methyl esters, as incorporated into the method for isolated *trans* and resubmitted to the Uniform Methods Committee. Three members would approve of this technique with certain specified modifications. Two members oppose the diazomethane method unless the $H_2SO_4$ method is approved as an alternate method (Rules of the Uniform Methods Committee do not permit alternate procedures). No views were obtained from the remaining two members. On the basis of this split in viewpoint, the Uniform Methods

Committee for the second consecutive year did not accept the procedure for isolated *trans*-isomers as a tentative method.

After considerable discussion of this situation, a modification of the diazomethane method described by Chipault for esterification of fatty acids on a small scale was adopted as the most satisfactory procedure by unanimous vote of the seven members present (5).

An abbreviated version of the experimental procedure will be incorporated into the method for isolated *trans*-isomers and, along with the changes to make the method directly applicable to long-chain fatty acids where the *trans* content is above 15%, will be revised and resubmitted to the Uniform Methods Committee. With these two additions the approved method is as published with last year's report (1).

*Other Committee Discussions and Future Planning.* Discussions of future activities of the Spectroscopy Committee were centered on investigations of the recently published methods involving the near-infrared region. Suggestions were made by various members that the committee collaboratively investigate near-infrared method for hydroxyl number, for epoxy value, and for direct determination of *cis*-isomers and the combination of such values with the established method for *trans*-isomers to afford an infrared method for total unsaturation. It was decided that recommended procedures for these determinations would be sent to the chairman and that during the coming year one or two of these methods be collaboratively tested for possible recommendation for establishment as tentative official methods.

### Cooperation with Coblentz Society

At a meeting in New Orleans, April 21, 1959, the Spectroscopy Committee decided that cooperation with the Coblentz Society in the collection and dissemination of infrared spectra would provide the simplest and most feasible means of making infrared spectra of fatty acids and their derivatives available to any member of the Society and to the entire fat and oil industry. It was decided that spectra should be submitted to the chairman of the Spectroscopy Committee, who is already acting as one of the collectors of infrared spectra for the Coblentz Society. The Society has endorsed this plan and furthermore has established a procedure whereby oil chemists can obtain a packet of the reproduced spectra of fatty acids and their derivatives without the requirement of subscribing to all the spectra issued by the Society. However, to date, no spectra have been received for submission to the Coblentz Society. The committee again urges all members throughout the A.O.C.S. to participate in this activity. Spectra may be submitted to the chairman of the Spectroscopy Committee, and details of the plan, requirements for spectra to be submitted, etc., may be obtained from him.

### Acknowledgment

The Spectroscopy Committee is aware that, particularly in the collaborative testing program, it is indebted to several individuals for assistance in making spectral measurement and computations and in offering suggestions. The services of these individuals is again gratefully acknowledged. The chairman, in particular, wishes to acknowledge the considerable assistance of Miss Elizabeth R. McCall in compiling, recomputing, and arranging the collaborative data and of E. Fred Schultz Jr. for the statistical analysis included in this report.

ROBERT R. ALLEN
J.R. CHIPAULT
ROBERT O. CRISLER
SAMUEL F. HERB
RALPH E. KELLY
WILLIAM E. LINK
B.N. ROCKWOOD
DONALD H. WHEELER
HANS WOLFF
ROBERT T. O'CONNOR, chairman

REFERENCES

1. A.O.C.S. Spectroscopy Committee 1958–59. J. Am. Oil Chemists' Soc., *36*, 627–631 (1959).
2. Mandel, John, Gordon Research Conference on Statistics in Chemistry and Chemical Engineering, July 21–25 (1958).
3. Mandel, John, and Lashof, T.W., ASTM Bull. *No. 239*, 53–61 (July 1959).
4. Mandel, John, Technometrics, *1*, 251–267 (August 1959)
5. Schlenk, Hermann, and Gellerman, Joanne L., Anal. Chem., *32*, 1412–1414 (1960).

# Comparison of Fatty Acid Esters of Sucrose and of Polyoxyethylene in Built Detergent Compositions[1]

**LLOYD I. OSIPOW and FOSTER D. SNELL, Foster D. Snell Inc., New York, New York**

The addition of sucrose monotallowate to an aqueous solution of alkanolamine alkylaryl sulfonate lowers the total active-agent content required for the formation of liquid crystals. In contrast, the addition of polyoxyethylene esters of fatty acids to the alkylaryl sulfonate solution increased the total amount of active agent required for the formation of liquid crystals. The deaggregating effect of the ethylene oxide-derived nonionics was reflected in reduced foam and detergency for combinations of these nonionics and alkylaryl sulfonate. Foam and detergency remained at a high level with combinations of sucrose monotallowate and alkylaryl sulfonate. Higher hydrophile-lipophile balance (HLB) values were obtained with the sugar esters than with the polyoxyethylene nonionics. The results were examined in terms of Winsor's theory of intermicellar equilibria.

[1] Presented before the 34th fall meeting, American Oil Chemists' Society, October 17–19, 1960, New York.

AN EXTENSIVE body of literature has been developed concerning the influence of cryoscopic forces on the properties of detergent solutions. The term "cryoscopic forces" as used here refers to the forces of cohesion and repulsion between amphipathic molecules that determine the degree of packing of these molecules in surface films and micelles.

Solutions of sodium lauryl sulfate give expanded monolayers. The addition of lauryl alcohol results in a condensed monolayer at the water-air interface, as evidenced by the tremendous increase in surface viscosity (1). The lauryl alcohol addition enhances foam stability (2) and detergency. Similar effects are observed upon the addition of fatty alkanolamides to solutions of sodium dodecylbenzene sulfonate (3).