are of high quality such as those evaluated in the previous report (2). A manufacturer using a concentrate of lower quality, i.e., one containing more irrelevant light-absorbing materials at 328 mμ and more irrelevant chromogenic materials in the $SbCl_3$ test, must establish for himself a correlation between biological and non-biological assay results before using the latter methods exclusively in controlling the vitamin A fortification of his product.


### Summary

A simple and precise spectrophotometric procedure has been described for the control of vitamin A fortification of margarine made in the plant. This method involves readings taken at 328 mμ of the whole margarine oil dissolved in cyclohexane. The spectrophotometer was set at 100% transmission with the corresponding unfortified oil at the same concentration in cyclohexane. Physico-chemical and biological assay data have been presented demonstrating that the spectrophotometric method is the most reliable procedure for assaying margarine for vitamin A following fortification with quality vitamin A concentrates. It is far more precise than the biological assay method and equally as specific for vitamin A when the fortifying concentrates used satisfy the requirements set forth in the preceding paper (2).

The colorimetric procedure, involving the reaction of vitamin A in the unsaponifiable extract of margarine with antimony trichloride, tends to overestimate on the average by about 600 USP units per pound the true vitamin A content of the margarine. However it is not at all unusual for the overestimates to exceed 1,000 USP units. The colorimetric method is useful as a screening test on open-market samples for estimating maximal vitamin A potencies and as a check on the reliability of the unfortified oil blanks used in the spectrophotometric assay. Reasons were given for the belief that some conversion of vitamin A to the biologically-inactive anhydro vitamin A occurs in margarine and that the presence of this derivative is responsible in large part for the discrepancy between colorimetric and spectrophotometric estimates.



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# Iodine Number Determinations by Dead-Stop Titrimetry

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THE methods for the determination of iodine numbers indicating the degree of unsaturation of fats and oils are well established analytical procedures. The routine procedure in our laboratory is that by Hanus (1, 2). The Wijs method (3) is recommended by the American Oil Chemists' Society. A recent development (4) modifies the Rosenmund-Kuhnhenn procedure by using a pyridine sulfate dibromide solution in conjunction with mercuric acetate as a catalyst. This reduces the reaction times to one minute for nonconjugated and 30 to 120 minutes for highly conjugated oils (5). The starch endpoint is employed throughout.

It was thought desirable to apply the amperometric (dead-stop) titration procedure (6) to the above methods and compare the results obtained, using a variety of fatty acids and oils which differ in their degrees of unsaturation.

### Apparatus

The instrument used in these determinations was the one previously described (6), modified in such a way that arbitrary readings on the galvanometer indicated the potential at the electrodes (see Figure 1). This secured a uniform potential and served to check the correct functioning of the instrument. A Fisher Model A galvanometer with a sensitivity of 0.10 microamps per mm. was employed.

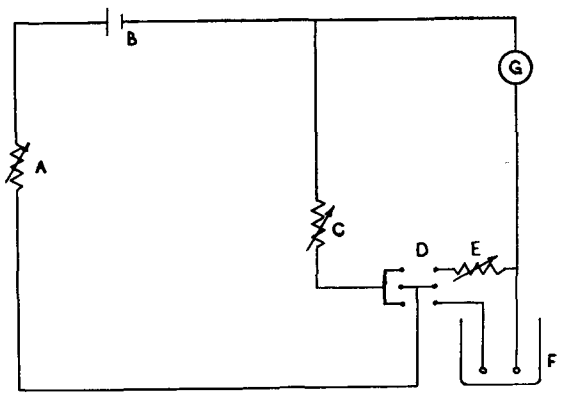


FIG. 1. Dead-stop titration apparatus.
A. 2 K-ohm variable resistor.
B. 1.5 volt dry cell.
C. 20-ohm variable resistor.
D. Double pole throw switch.
E. 5 K-ohm variable resistor.
F. Pt. electrodes in cell (coiled platinum wire).
G. Galvanometer.

[1] Communication No. 249.

A Leeds and Northrup potentiometer (Type K2) was used to calibrate the instrument. The 2 K-ohm resistance was calibrated in 25 mv. intervals and the 20 ohm in 5 mv. intervals. The 5 K-ohm resistor was so set that the galvanometer showed half-scale deflections for 10 mv. increments from 10 mv. to 100 mv. A double-pole throw switch connected this circuit whenever knowledge of the magnitude of the potentials across the electrodes was desired.

## Experimental

Preliminary experiments were conducted to define the optimum conditions for the titrations. Tests showed that none of the chemicals used (glacial acetic acid, pyridine, sulfuric acid, and mercuric acetate) adversely affected the polarization phenomena upon which the Foulk and Bawden procedure depends.

In order to determine the optimum voltage, titrations with blank reagents were performed at three different voltages. From Figure 2 it can be seen that the titrations are most feasible with a 10 mv. potential across the electrodes. A higher potential gives too large a galvanometer deflection per drop of thiosulfate solution added while a lower one has the opposite effect. It can also be noticed from the curves presented that towards the end of the titrations the curves straighten out. With a 10 mv. potential it is necessary to take only two or three galvanometer readings below midscale and then extrapolate the straight line to a zero reading. This is the procedure followed in the actual iodine value determinations.

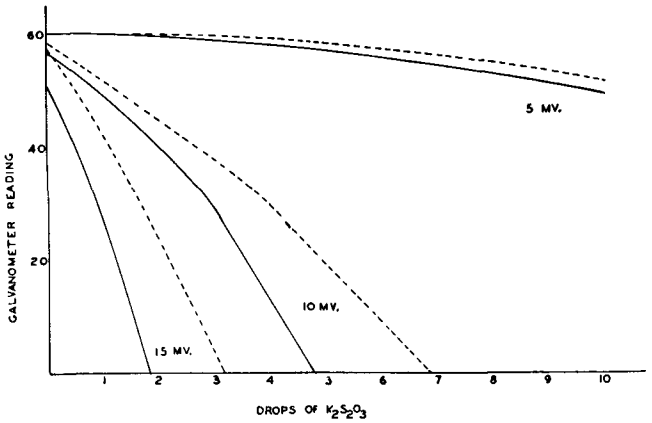


FIG. 2. Blank titration curves.
———— Hanus reagents.
– – – – – – – Benham-Klee reagents.

It was seen moreover that there existed an optimum concentration range for the titration. When blank titrations were performed with the Hanus reagents, an addition of 100 ml. of water resulted in the best balance between galvanometer readings and drops of thiosulfate reagent added. In Figure 3 are recorded the results of this experiment.

It was evident when applying a wide variety of stirring speeds that this factor has no noticeable effect on the titration. However a minimum stirring speed sufficient to maintain a satisfactory partition of iodine between the solvent layer and the aqueous phase is required.

In the determinations of the iodine values, commercial oils or fatty acids were used with two exceptions. The oleic acid was purified according to the method of Brown and Shinowara (7). The description of the preparation of the *Fusarium lini* Bolley (F1B) urea adduct filtrate oil is in press (8).

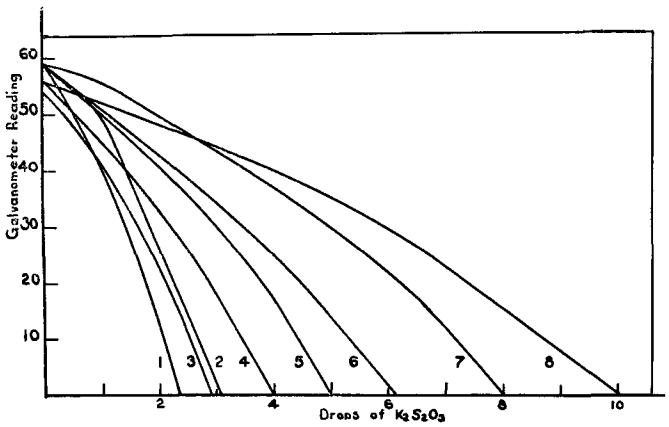


FIG. 3. Effect of volume on titration curves. All contain 10 ml. of chloroform, 15 ml. of Hanus reagent, and 10 ml. of potassium iodide solution. Voltage used was 10 mv.

1. 50 ml. of water.
2. 75 ml. of water.
3. 100 ml. of water.
4. 150 ml. of water.
5. 200 ml. of water.
6. 250 ml. of water.
7. 300 ml. of water.
8. 400 ml. of water.

With the three methods the values obtained by means of the instrument were compared to those obtained by the unmodified procedures, using starch. In our analyses the electrodes were lowered into the reaction flask, and the potential was adjusted to 10 mv. The thiosulfate solution was added until the galvanometer needle swung violently. This gave a clear indication of the approach of the end-point. When the galvanometer read approximately half-scale, the burette reading was taken. Two more sets of galvanometer-burette readings were taken, and the values were plotted. The position at which the straight line drawn through the three points cut the abscissa was taken as the stoichiometric end-point.

## Conclusions

The iodine values obtained with the starch and the dead-stop procedure with the various methods used are listed and compared in Table I. It is evident that the reliability of the instrument values cannot be questioned. On the contrary, we believe that these values approach more closely the true stoichio-

TABLE I

Comparison of Iodine Values by Dead-Stop Titrimetry and by Starch

| Oil or fatty acid | Iodine value by instrument | Iodine value with starch |
|---|---|---|
| **Hanus Method** | | |
| Peanut oil | 89.87 | 89.30 |
| Cottonseed oil | 102.9 | 102.4 |
| Corn oil | 124.4 | 124.4 |
| Soybean oil | 135.5 | 135.0 |
| **Benham-Klee Method** | | |
| Oleic acid | 89.40 | 89.18 |
| Linoleic acid | 177.5 | 176.1 |
| Linseed fatty acids | 177.8 | 177.3 |
| F1B urea adduct filtrate oil | 183.2 | 182.6 |
| **Wijs Method** | | |
| Coconut oil | 10.18 | 10.13 |
| Olive oil | 74.61 | 74.06 |
| Rapeseed oil | 104.3 | 104.1 |
| Peach kernel oil | 105.0 | 104.3 |

metric endpoint of the titrations and consequently represent the true iodine numbers of the unsaturated substance because of a) the absence of the subjective factor in fixing the disappearance of the starch-iodine blue color, b) the impossibility of using an excess of thiosulfate solution (the endpoint is not actually reached with the thiosulfate reagent but is calculated from an extrapolation to a point of zero potential), and c) the absence of errors arising from adsorption phenomena, such as occur in the iodine-starch system.

A useful application of dead-stop titrimetry could be found in micro iodine value titrations. It has been demonstrated (Foulk and Bawden) that in titrating iodine with a thiosulfate solution, the endpoint, as shown by the instrument, occurs 4 drops of 0.001N thiosulfate solution after the blue starch-iodine color disappears. This is probably due to the removal of iodine from the titration by adsorption on the starch. The value of the instrument in micro iodine number determinations is obvious.


### Summary

The amperometric (dead-stop) method has been applied to iodine value determinations of fatty acids and oils. The values obtained agree with those arrived at by the starch end-point method. The use of this simple instrument appears to give however more accurate results since the endpoint is determined by the extrapolation of the linear response of the galvanometer with small increments of added thiosulfate to a zero galvanometer reading which corresponds to a zero iodine concentration. The fluctuations of the galvanometer needle give visible evidence of the approach of the endpoint, thereby facilitating the titration.



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# Dilatometric Studies of Pure Triglycerides and Mixtures[1,2,3]

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THE volume-temperature relationships of various natural fats and some synthetic triglycerides have been studied with volumetric and gravimetric dilatometers by several investigators. Coffey and Spannuth (4) employed a simple volume dilatometer to determine these relationships in various fats and fat mixtures having widely different fatty acid compositions and found that the measurements in a general way reflected the fatty acid composition of such systems. Bailey and his co-workers (2,3,6,8) employed a gravimetric dilatometer to measure the volume changes associated with phase transformations, the coefficients of expansion in the solid and liquid states, and the melting dilations for several simple triglycerides. They calculated the percentage of solids at various temperatures for several fats and fat mixtures from dilatometric data on the assumption that all triglycerides present in such mixtures possessed equal melting dilations. The results appeared to be in agreement with calorimetric data which they had previously obtained.

Inasmuch as the melting dilations of individual triglycerides have been shown to vary quite considerably, depending on their fatty acid composition, Bailey (1) has recently indicated that the exact calculation of the proportion of the solid and liquid phases in the complex mixtures that constitute natural fats is impossible. This is also true of a simple binary mixture of triglycerides unless *a priori* knowledge of the identity, proportions, and properties of the individual components is available.

The work reported here was undertaken to extend the available information on the coefficients of expansion and melting dilations of individual triglycerides and to determine the effects of triglyceride composition on the melting range and on the proportion of solids within the melting range in synthetic mixtures of pure triglycerides.

### Materials

Eight simple and mixed triglycerides were employed. The pure symmetrical mixed triglycerides, oleodistearin, oleodipalmitin, stearodiolein, palmitodistearin, and stearodipalmitin were prepared as described in another paper (5). The simple triglycerides, tristearin, tripalmitin, and triolein were prepared by reacting purified methyl esters of the fatty acids with triacetin using sodium methoxide as a catalyst (7). The properties of these simple triglycerides agreed with those reported in the literature for the pure compounds.

Various mixtures of the simple and mixed triglycerides containing two, three, four, and eight components were prepared. The mixtures of triglycerides examined contained 33⅓ mol per cent of saturated fatty acids and 66⅔ mol per cent of oleic acid.

### Apparatus and Methods

A gravimetric type of dilatometer was used, similar to that described by Bailey and Kraemer (2), and is

[1] Paper No. 2703 of the Scientific Journal Series, Minnesota Agricultural Experiment Station, and Hormel Institute publication No. 73.
[2] Submitted as part of Ph.D. Thesis by B. M. Craig, University of Minnesota, 1950.
[3] Presented at meeting of the American Oil Chemists Society, San Francisco, Calif., September, 1950.