# The Determination of Free Hydroxyl Groups in Fatty Materials

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HYDROXYL GROUPS of greatest analytical interest to the fats and oils chemist are associated with hydroxy acids, fatty alcohols, monoglycerides, diglycerides, and polyalcohols like glycerine. The most important hydroxy acid is ricinoleic acid, which constitutes nearly 90% of the fatty acids present in castor oil. Its commercial importance arises from the fact that the readily effected elimination of a molecule of water from castor oil acids converts the acid to conjugated linoleic acid, a polymerizable material of great interest in the drying oil industry.

Fatty alcohols like lauryl alcohol, made by the catalytic reduction of lauric acid, are extensively used in detergent formulations. Monoglyceride emulsifiers are produced in large volume for use in shortenings, cosmetics, etc. All of these compounds are characterized by having free hydroxyl groups present in their structures. Such free hydroxyl groups, irrespective of their location in the molecule, react quantitatively with acetic anhydride.

Two established analytical procedures are available for making routine use of this chemical reaction. The standard A.O.C.S. procedure, often called the André-Cook method (1, 2, 3), has been in use for many years and is carried out in the following manner. A dry sample of fatty material is refluxed with an excess of acetic anhydride for an appropriate length of time. The excess acetic anhydride and the acetic acid formed are eliminated from the reaction mixture by repeated washing with hot water. Determinations of saponification value are then made on samples of the acetylated (washed and dried) and unacetylated fat. The extra potassium hydroxide needed to saponify the acetylated sample as compared with the original material constitutes the alkali required to neutralize the acetic acid derived from the acetate groups attached to the free hydroxyls. A direct relationship exists between the number of hydroxyl groups in the sample and the number of equivalents of acetic anhydride used in the acetylation step and the equivalents of potassium hydroxide required to neutralize the acetic acid derived by hydrolysis of the acetylated sample.

André and Cook preferred to express the results of this determination as the Acetyl Value, which was defined as the number of milligrams of potassium hydroxide required to neutralize the acetic acid obtained by saponifying one gram of acetylated oil.

By definition S = mg. KOH to saponify 1 g. of an oil with "e" ester groups and a molecular weight of "m"

$$S = \frac{56e \times 1000}{m} \qquad (1)$$

If this oil has "n" OH groups and is acetylated, the acetylated oil will have a molecular weight of "m'" and a saponification number of "S'." Since on saponification the acetate is saponified off, as well as the "e" ester groups

$$S' = \frac{(56e + 56n)\ 1000}{m'} \qquad (2)$$

Since for each OH group, acetylation adds 42 to the molecular weight

$$m' = m + 42n \qquad (3)$$

The acetyl value "A" is defined as the mg. of KOH required to neutralize the acetic acid obtained from 1 g. of acetylated oil

$$A = \frac{56n \times 1000}{m'} = \frac{56{,}000n}{m'} \qquad (4)$$

Eliminating 56e between *(1)* and *(2)*

$$m'S' = Sm + 56{,}000n \qquad (5)$$

From *(3)* and *(5)* m can be eliminated

$$m'S' = Sm' - Sn42 + 56{,}000n \qquad (6)$$

Rearrange *(6)* and substitute for m' from *(4)*

$$(S' - S)\frac{56{,}000n}{A} = 56{,}000n - Sn42 \qquad (7)$$

Factor out n, and consolidate *(7)*

$$S' - S = A\left(1 - \frac{42S}{56{,}000}\right) = A\ (1 - .00075S) \qquad (8)$$

$$A = \frac{S' - S}{1 - .00075S} \qquad (9)$$

If it is desired to express the results in terms of the hydroxyl value H, equation *(4)* becomes

$$H = \frac{56n \times 1000}{m} \qquad (10)$$

since H is defined as the number of mg. of KOH equivalent to the hydroxyl content of the sample based on the weight of unacetylated fat.

Then, from *(3)* and *(5)* m' is eliminated

$$mS' + 42nS' = Sm + 56{,}000n \qquad (11)$$

Rearrange *(11)* and substitute for m from *(10)*

$$(S' - S)\frac{56{,}000n}{H} + 42nS' = 56{,}000n \qquad (12)$$

$$S' - S = H\left(1 - \frac{42\,S'}{56{,}000}\right) = H\ (1 - .00075S') \qquad (13)$$

$$H = \frac{S' - S}{1 - .00075S'} \qquad (14)$$

From *(9)* and *(14)* and *(3)*

$$H/A = \frac{m'}{m} = \frac{1 + 42n}{m} \qquad (15)$$

From *(10)*

$$H/A = 1 + \frac{42\,nH}{56{,}000n} = 1 + .00075H \qquad (16)$$

Hence

$$A = \frac{A}{1 + .00075H} \qquad (17)$$

and

$$H = \frac{H}{1 - .00075A} \qquad (18)$$

THIS approach to the estimation of the hydroxyl value is quite tedious and time-consuming. Care must be exercised to insure complete acetylation. The acetylated sample must be thoroughly washed and dried before the saponification value determination is made. Reliable hydroxyl value estimates require a high degree of precision in making the saponification value determinations.

West, Hoaglund, and Curtis ( 4) devised a quicker, less tedious, and more direct method. In this case a suitable excess of a pyridine solution of acetic anhydride is used to acetylate the sample. The excess of acetic anhydride remaining after acetylation of the hydroxyl groups in the sample is hydrolyzed to acetic acid with water and determined by titration with standard potassium hydroxide solution. The difference between this titration and a reagent blank titration represents the amount of potassium hydroxide equivalent to the acetic anhydride absorbed by the hydroxyl groups in the sample. Because most fat samples usually contain some free fatty acids and occasionally some free alkali, it is necessary to titrate the sample itself with standard alkali or acid to furnish an appropriate correction factor.

**West, Hoaglund, and Curtis Formula**

$$\text{Hydroxyl value} = \frac{\left[\left(B + \frac{WA}{C}\right) - D\right] N(56.1)}{W}$$

A = ml. KOH solution required for acid value blank
B = ml. KOH solution required for reagent blank
C = weight of sample used for acid value blank
D = ml. KOH solution required for titrating acetylated sample
N = normality of KOH solution
W = weight of sample used for acetylation

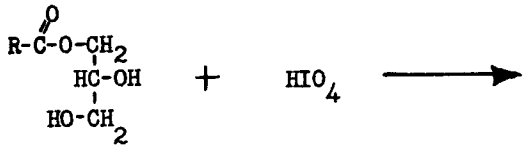
It is necessary to know the approximate hydroxyl value of the sample when using this method because the excess of acetic anhydride employed in the acetylation step is critical. If more than 70% of the reagent is absorbed by the sample, the assay results will probably be in error. A repeat determination must then be made, using a more suitable ration of sample weight to reagent amount. The method is not effective when used on samples like glycerine, which have very high hydroxyl values because the small sample weights required result in poor reproducibility. Primary and secondary amines interfere with the analysis by tying up some acetic anhydride. This source of error must be guarded against when applying the method to fatty alcohols extracted from detergent mixtures which also contain amino compounds.

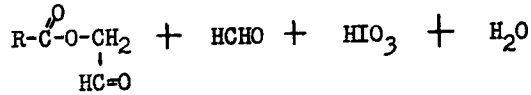
The obvious advantages associated with the West, Hoaglund, and Curtis method are such that it has largely replaced the André-Cook procedure. Collaborative work is in progress in the American Oil Chemists' Society and in the American Society for Testing Materials which will soon lead to the adoption of suitably modified versions of the West, Hoaglund, Curtis procedure as official methods.

Hydroxyl values obtained in this way are of limited value because they represent all hydroxyl groups irrespective of their location in the fat molecule. The presence of mono- and diglycerides and/or glycerine in castor oil, for example, would lead to erroneously high ricinoleic acid estimates. The method does not permit discrimination between mono- and diglyceride hydroxyl groups. This particular deficiency was a very important handicap in evaluating commercial monoglyceride concentrates which contain both of these compounds, although their effectiveness is for the most part associated with the monoglyceride concentration only.

FOR a while before Pohle, Mehlenbacher, and Cook (6) published their important adaptation of Malaprade's (5) periodic acid oxidation reaction, which was specific for monoglycerides as compared with diglycerides, potency or value of monoglyceride concentrates was often characterized by interfacial tension measurements (7).

Calibration curves were constructed by plotting interfacial tension measurements taken in oil-water interfaces against the concentration of representative monoglyceride concentrates in the oil phase. This technique was quite useful but lacked the sensitivity and specificity subsequently made available by the Pohle, Mehlenbacher, and Cook method.





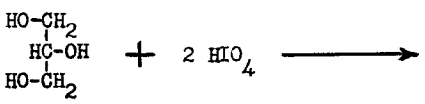
In an acid medium and at moderate temperatures this reaction is quantitative and quite specific to compounds having two or more adjacent hydroxyl groups.

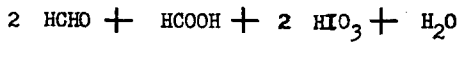
High results were often obtained when this method was applied to solid-at-room-temperature concentrates because the heat applied to melt the sample in order to insure adequate contact with the reagent increased significantly the magnitude of the side oxidation reactions. This difficulty was substantially eliminated by dissolving the solid concentrate in a suitable solvent before adding the periodic acid reagent (8). The sample solution and reagent are shaken in a suitable mechanical shaker for an appropriate length of time to insure completion of the reaction (2 min.). Potassium iodide solution is then added, and the liberated iodine is titrated with standard thiosulphate solution. The difference between this titration and a reagent blank titration represents the amount of the reagent used up in oxidizing the sample. Before the monoglyceride concentration of the sample can be calculated in the conventional way, it is necessary to obtain the average molecular weight of the sample by determining its saponification value.

The presence of free glycerine in samples to which this method is applied will cause the monoglyceride

content to be grossly overestimated on occasions. Glycerine contains three adjacent hydroxyl groups which act as a pair of two hydroxyls each so far as the reaction with periodic acid is concerned. In these circumstances and also due to the difference in molecular weight between glycerine and mono-olein, for example, each percentage of glycerine present in the sample would add about 7.5% to the mono-olein estimate. It is possible to extract the glycerine from the monoglyceride mixture, but the method is tedious, time-consuming, and difficult. Care must be observed to avoid loss of monoglyceride in the wash water. Optimum results were obtained when the sample was dissolved in ethyl acetate and then washed repeatedly with a fairly strong salt water solution. The ethyl acetate used to dissolve the sample and salt in the water tend to minimize monoglyceride loss during the glycerine extraction.

FORTUNATELY it is no longer necessary to resort to this tedious extraction technique. The oxidation of glycerine with periodic acid yields one equivalent of formic acid and two equivalents of formaldehyde.





Formaldehyde Formic Acid

No formic acid is formed when monoglycerides are oxidized with periodic acid. It is quite a simple matter to determine the amount of formic acid formed by titration with standard alkali and from this data to calculate the concentration of glycerine in the sample. The monoglyceride estimate can then be corrected to eliminate the effect of the glycerine present in the sample.

Periodic acid reacts with glycerine in neutral solution whereas a strongly acid medium is necessary for its reaction with monoglycerides. Within the limits of its solubility potassium periodate can be used in place of periodic acid to eliminate the need for a reagent blank. The formic acid titration end-point is often slow and indistinct due to the buffering action of iodic acid. The Miner Laboratories (12) have discovered that if a water solution of the periodic acid reagent is contacted with the sample dissolved in chloroform, an iodine-sodium thiosulphate titration can be used to eliminate the above mentioned end-point trouble.

Pohle and Mehlenbacher (9) were the first to take advantage of this unique oxidation behavior of glycerine when analyzing mixtures containing glycerine, propylene glycol, and trimethylene glycol. The propylene glycol responds to the periodic acid reagent like monoglycerides, and the trimethylene glycol is unaffected. The authors made a total hydroxyl value determination on the sample, using the West, Hoaglund, and Curtis method previously described, and with this additional data were able to calculate the trimethylene glycol concentration, completing the analysis of the mixture.

Troy and Bell (10) made use of this same principle to determine the relative amounts of glycerol, diglycerol, and polyglycerol in commercial diglycerol mixtures. Polymerized glycerine behaves like monoglycerides in the periodic acid oxidation and yields no formic acid because only two adjacent hydroxyl groups are involved.

The appropriate factor for calculating the percentage of diglycerol was determined by analysis of several pure diglycerol samples prepared by vacuum distillation of typical commercial mixtures. This step is necessary because there is no method available for distinguishing between the $\alpha,\alpha$ diglycerol, which absorbs two equivalents of periodic acid, and the $\alpha,\beta$ diglycerol, which only requires one equivalent of the oxidizing reagent. The reliability of this method is closely associated with the consistency of the process in producing about the same relative amounts of the two diglycerol isomers each time.

The same authors devised a useful method for obtaining a substantially complete analysis of commercial monoglyceride concentrate mixtures (11). After obtaining free glycerine and monoglyceride estimates in the prescribed manner, Troy and Bell saponified the sample and obtained a total glycerine assay. When suitable account has been taken of the free glycerine and the monoglyceride glycerine, the remaining glycerine can be divided between diglycerides and triglycerides in only one way for a given molecular weight of fatty acids.

$$\text{Percentage of combined glycerine} = \frac{100 - (\%\ \text{monoester} + \%\ \text{diester})}{\text{Mol. wt. triester}} \times 92.09 + \frac{\%\ \text{Diester}}{\text{Mol. wt. diester}} \times 92.09 + \frac{\%\ \text{Monoester}}{\text{Mol. wt. monoester}}$$

In this equation the % diester is the only unknown, consequently its value can be readily calculated by filling in the known values and solving the equation. The concentration of the triester can then be obtained by subtracting the sum of the other predetermined constituents from 100%.

Methods have been described which permit reasonably accurate and precise estimation of total hydroxyls, monoglyceride hydroxyls, and glycerine hydroxyls. By suitable application of these techniques, mixtures containing all three types of hydroxyl groups can be adequately resolved.

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