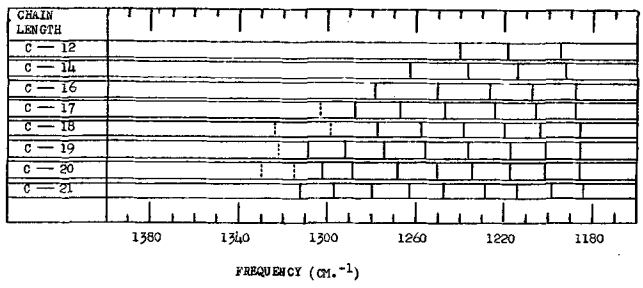
vibrations of the methylene group. Furthermore these bands are not seen in the spectra of the fatty acids in $CS_2$ solution. As the carbon chain increases in length, the number of recognizable progression bands increases from 3 in the case of lauric acid to 9 in the case of heneicosanoic acid (Table 8). The increase in the number of bands with chain lengths, the regularity of the spacings, and the displacement to lower frequencies with increasing chain lengths are clearly depicted in Table 8. With more quantitative data along the same lines it should be possible to establish the chain length of binary, ternary, or polynary mixtures (31).

TABLE VIII




Both chemical and physical means are therefore available for the measurement of chain lengths. The accuracy of these measurements is however dependent on the purity of the fatty acids examined. As it is difficult to completely separate fatty acids of varying chain lengths from each other, it is apparent that accuracy is more dependent on a method for isolating them than on accurate methods of analysis. In spite of these difficulties it is apparent from this presentation that the measurement of chain length will become an important factor in the development of better shortenings and frying oils.

REFERENCES

1. Bailey, A. E., "Industrial Oil and Fat Products," 2nd ed., Interscience Publishers Inc., New York (1951).
2. Kummerow, F. A., Pan, H. P., and Hickman, H., J. Nutrition, *46*, 489 (1952).
3. Production Survey, The National Potato Chip Institute, Cleveland 15, Ohio (1953).
4. Smith, H. L., and Freeman, W. E., Food Eng., *27*, No. 3, 60 (1955).
5. Bailey, A. E., "Melting and Solidification of Fats," Interscience Publishers Inc., New York (1950).
6. Hilditch, T. P., "The Chemical Constitution of Natural Fats," J. Wiley and Sons Inc., New York (1947).
7. Kartha, A. R. S., "Studies on the Natural Fats," Keralamitram Press, Maharaja's College, Ernakulam (1951).
8. Bhalerao, V. R., and Kummerow, F. A., J. Dairy Sci., *37*, 156 (1954).
9. Foreman, H. D., and Brown, J. B., Oil and Soap, *21*, 183 (1944).
10. Schlenk, H., and Holman, R. I., J. Am. Chem. Soc., *72*, 5001 (1950).
11. Sato, Y., Barry, G. T., and Craig, L. C., J. Biol. Chem., *170*, 501 (1947).
12. Fitelson, J., J. Am. Oil Chemists' Soc., *47*, 1 (1950).
13. Bertram, S. H., Z. Untersuch. Lebensm., *55*, 179 (1928).
14. Official and Tentative Methods of the Am. Oil Chemists' Soc., Cd 5-40 (1946).
15. Hunziker, D. F., "The Butter Industry," published by D. F. Hunziker, La Grange, Ill. (1940).
16. Keeney, M., Ice Cream Rev., *38*, 6:85 (1955).
17. Keeney, M., Ice Cream Trade Journal, *49*, 2130 (1953).
18. Täufel, K., Fette u. Seifen, *52*, 10 (1949).
19. Ashley, B. D., and Westphal, U., Arch. Biochem. and Biophysics, *56*, 1 (1955).
20. Zbinovsky, V., Anal. Chem., *27*, 764 (1955).
21. Inouye, Y., and Noda, M., J. Agr. Chem. Soc. of Japan, *24*, 291 (1951).
22. Piper, S. H., Chibnall, A. B., and Williams, E. F., Biochem. J., *28*, 2175 (1934).
23. Francis, F., Collins, F. J. E., and Piper, S. H., Proc. Roy. Soc. Lond., *158A*, 691 (1937).
24. Francis, F., Piper, S. H., and Malkin, T., *ibid.*, *128A*, 214 (1930).
25. "Fatty Acids," by K. S. Markley, P83, Interscience Publishers Inc., N. Y. (1947).
26. Chibnall, A. C., Piper, S. H., Pollard, A., Williams, E. F., and Sahai, P. N., Biochem. J., *28*, 2189 (1934).
27. Piper, S. H., J. Soc. Chem. Ind., *56*, 61 (1937).
28. Rusoff, I. I., Platt, J. R., Klevens, H. B., and Burr, G. O., J. Am. Chem. Soc., *67*, 673 (1945).
29. O'Connor, R. T., Field, Elsie T., and Singleton, W. S., J. Am. Oil Chem. Soc., *28*, 154 (1951).
30. Sinclair, R. G., McKay, A. F., and Jones, Norman R., J. Am. Chem. Soc., *74*, 2570 (1952).
31. Jones, Norman R., McKay, A. F., and Sinclair, R. G., *ibid.*, *74*, 2575 (1952).

# Determination of Unsaturation

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ONE OF THE most important analytical determinations that an oil chemist must make is the measurement of unsaturation of an oil. Most manufacturing processes involve this determination to some extent as well as does the classification of fats and oils for trade and use. In spite of the importance of this determination there is as yet no one simple, rapid, and accurate method which will give consistent results on all types of oils. Much research time and effort has been expended to develop a good method, but the problem is yet unsolved. However, there are methods in use that do give a more or less quantitative measure of unsaturation; it is the purpose of this paper to discuss some of these methods.

The generally accepted method of expressing the degree of carbon to carbon unsaturation of a fat, oil, or derivative is by the term iodine value or iodine number. This value is defined as the grams of iodine which add to 100 g. of the sample, or in other words, the weight percentage of iodine based on the weight of the sample which adds to the sample. The results are expressed in terms of iodine whether iodine, or some other reagent, is actually used. Either of the terms, iodine value or iodine number, is correct; iodine value is preferred by the author and will be used in this discussion.

Since halogens will add to carbon-carbon double bonds, most of the methods make use of this property. In its simplest terms the determination consists of adding a halogen to a weighed quantity of sample and measuring in some way the amount of halogen which reacts with the unsaturation. The actual procedure for the determination is very simple. A small sample is weighed into a flask fitted with a ground glass stopper and is then dissolved in chloroform or carbon tetrachloride. A quantity of the halogen solution, usually iodine bromide or chloride in acetic acid, is pipetted into the flask containing the sample and also into a similar flask containing no sample (the blank). The flasks are stoppered and allowed to stand in the dark for at least 30 min. to allow the halogenation reaction to go to completion. An aqueous solution of potassium iodide is added and the halogen which failed to react with the sample reacts with the KI liberating iodine.

$$X_2 + 2KI = 2KX + I_2$$

The iodine is then titrated with standard sodium thiosulfate solution, using starch as an indicator.

$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6$$

$$I \rightleftharpoons Na_2S_2O_3$$

The blank is also titrated, and the iodine value of the sample is calculated according to the equation

$$\text{I.V.} = \frac{(B - S) \cdot N \cdot 12.69}{\text{wt. of sample}}$$

B = titration of blank
S = titration of sample
N = normality of $Na_2S_2O_3$ soln.

Many different halogen solutions have been proposed as the halogenating agent; only those which have gained acceptance will be mentioned.

The original method of determining the iodine value was described by Von Hübl (11), who used an alcoholic solution of iodine and mercuric chloride for the halogenation of the sample. The active reagent is actually iodine monochloride. A reaction time of 18 hrs. was necessary, and the reagent had very poor keeping quality because of the reaction of iodine monochloride and water. To overcome this difficulty Wijs (12, 13) used a solution of iodine monochloride in glacial acetic acid as the halogenating agent. This method is today accepted as one of those most satisfactory for the determination of isolated unsaturation. The Hanus method (4), which employs iodine bromide in acetic acid, is also fairly well accepted although it is reputed to give slightly low results. Rosenmund and Kuhnhenn (10) proposed the use of a solution of pyridine sulfate and bromine in glacial acetic acid. Bromine in a saturated methyl alcoholic solution of sodium bromide was proposed by Kaufmann (5) but has not gained wide acceptance. There are several variations of each of these methods, such as the use of a mercuric acetate catalyst to shorten the reaction time (1, 6).

These methods will give a fairly quantitative measure of unsaturation if the double bonds are not conjugate with each other or a carbonyl oxygen and if the determination is carried out under very specific conditions as to the excess of halogen reagent, time of reaction, and exclusion of light.

It might be well at this point to examine the mechanism of halogen addition to double bonds and to suggest some of the shortcomings of the methods.

In a polar solvent the halogen molecule is thought to be more or less polarized or, in other words, there is a separation of the charges within the molecule.

$$Br\text{-}Br \underset{\text{solvent}}{\rightleftharpoons} Br^{+}\text{–}Br^{-}$$

This polar halogen can now attack an ethylenic bond which, because of the influence of the polarized halogen, tends to shift the electrons of the double bond to one carbon, thus facilitating attack by the positive charged halogen.

$$R_1\text{-}\overset{H}{\underset{}{C}}=\overset{H}{\underset{}{C}}\text{-}R_2 + Br^{+}\text{–}Br^{-} \rightleftharpoons R_1\text{-}\overset{H}{\underset{+}{C}}\text{–}\overset{H}{\underset{}{C}}\text{-}R_2 \quad (Br^{-}\text{–}Br)$$

Depending on the concentration, solvent, and type of halogen involved, the halogen-halogen bond may break with formation of a covalent carbon-halogen bond (an over-all bimolecular reaction) or to complete the reaction collision with another halogen molecule may be necessary (a trimolecular reaction).

Although the reaction is reversible (2), the equilibrium lies far to the right, and when an excess of halogen is used, the reaction may be considered essentially quantitative. It has been shown (2) that the addition of potassium iodide solution after the reaction of iodine monochloride could lead to the following reactions

$$>C(I)\text{–}C(Cl)< + KI \rightarrow >C(I)\text{–}C(I)< + KCl$$

$$>C(I)\text{–}C(I)< \rightleftharpoons >C{=}C< + I_2$$

resulting in low iodine values. However this has not been shown to occur to any great extent during the determination.

Substitution reactions are also a source of error. Since this reaction is catalyzed by light, the exposure of the reaction mixture to a strong light during the halogenation must be kept at a minimum. Although undoubtedly some substitution takes place, it does not seem to be the source of a very large error in the usual iodine value determination.

From the studies of the kinetics and the mechanism of the halogenation reactions it is evident that, by the use of an excess of halogen, polar solvent, and catalysts, the reaction time is shortened, and the effect of the equilibrium reversal is minimized. Also the shorter reaction time should minimize the substitution reaction.

The addition of halogen to a conjugated system of double bonds is believed to occur first in the 1,4 position with a subsequent shift of the double bond.

$$R_1\text{-}\overset{H}{\underset{+}{C}}{=}\overset{H}{C}\text{–}\overset{H}{C}{=}\overset{H}{\underset{-}{C}}\text{-}R_2 + X^{+}\text{–}X^{-} \rightarrow R_1\text{-}\overset{H}{\underset{X}{C}}\text{–}\overset{H}{C}{=}\overset{H}{C}\text{–}\overset{H}{\underset{X}{C}}\text{-}R_2$$

The first mole adds rapidly, but the second mole adds very slowly because of the influence of the added halogens on the remaining double bond. Thus in the usual iodine value determination, low and variable results are obtained except when excessively long reaction times are employed. To overcome this Benham and Klee (1) proposed using the Rosenmund-Kuhnhenn reagent and a mercuric acetate catalyst for the determination of conjugated unsaturation. However it has been shown that the order of addition of the reagents has an effect on the determination. If the mercuric acetate catalyst is added before the halogen solution, better results are obtained (9). Exposure to light also effects the determination to some extent so it has been recommended that "low actinic" glassware be used in a darkened laboratory.

Quantitative hydrogenation is a method of measuring unsaturation which overcomes the objectionable features of the halogen method. However hydrogenation has several disadvantages among which are the time required, the more elaborate equipment needed, and the care necessary in its operation. For research purposes the hydrogenation method is to be recommended because the position of the double bonds has no effect on the determination, and substitution reactions are excluded. Essentially, the method consists of the catalytic hydrogenation of a sample and measurement of the quantity of hydrogen which reacts with the unsaturated bonds. The results can be expressed as moles of hydrogen per mole of sample or calculated to an iodine value basis; the latter is known as the hydrogen-iodine value.

The apparatus necessary to carry out the hydrogenation reaction consists of a reaction flask so fitted

as to allow introduction of the sample. The flask is connected to a burette to measure the hydrogen taken up by the sample and a levelling bulb to adjust the pressure in the apparatus. Several excellent types of apparatus have been described (7, 8). Figure 1

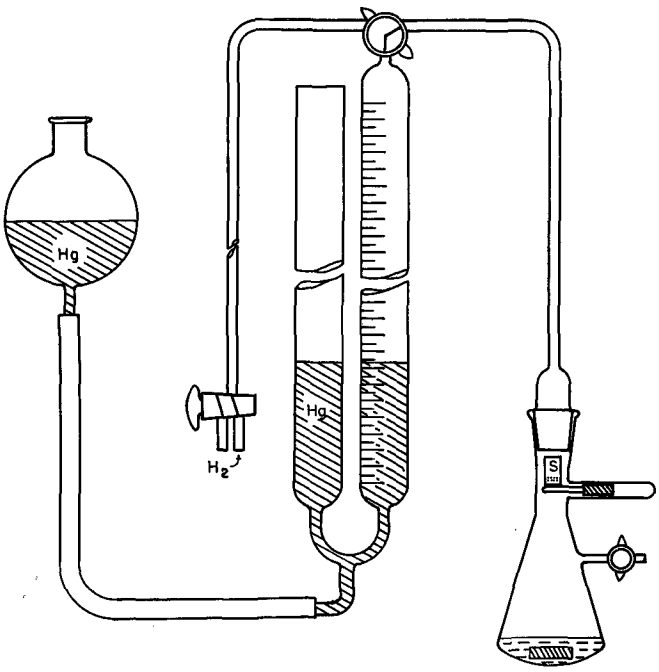


FIG. 1. Apparatus for quantitative hydrogenation.

shows one which we are using in our laboratory. In use, a sample which will take up about 30 ml. of hydrogen is weighed into a small cup; the solvent, usually 10 ml. of ethyl alcohol, a small amount of catalyst, and the magnetic stirring bar are placed in the reaction flask and the sample placed on the sample holding bar. The flask is then connected to the burette. The stopcocks on the flask and on the burette are opened, and hydrogen which has been saturated with solvent is allowed to flow through the system. The catalyst may also be reduced at this time by stirring. After the system has been swept by hydrogen for some time, the stopcock on the flask is closed and the stopcock on the burette turned to fill the burette with hydrogen. The gas flow is stopped, and the stopcock is turned to connect the reaction flask with the burette. The reduction of the catalyst is completed, the system allowed to come to equilibrium, and the levelling bulb adjusted until the the pressure is equalized. The burette reading, temperature, and pressure are noted. The sample is dropped into the solvent-catalyst and stirred until no more hydrogen is taken up, as indicated by a constant volume reading of the burette. The burette reading, temperature, and pressure are noted as before. The volume of hydrogen at S.T.P. may be calculated by the equation

$$V_1 \text{ or } V_2 = \frac{P - P_s}{760} \cdot \frac{273}{T} \cdot V_a + V_B$$

where $V_1$ and $V_2$ are STP volumes before and after hydrogenation of the sample, P = atmospheric pressure, $P_s$ = vapor pressure of the solvent at temperature of the apparatus T. $V_a$ is the volume of the reaction flask to the top of the burette, and $V_B$ is the burette reading. The hydrogen-iodine value = $(V_1 - V_2)$ (25384)/22412 · weight of sample. Platinum oxide is a very good catalyst for the hydrogenations but must be reduced in the apparatus. A supported palladium catalyst may be prereduced, which gives much lower blank determinations. The determination may be carried out in about 30 min.

The problem of control of commercial hydrogenations is one that calls for a very rapid method for the determination of unsaturation. The method that is now used to a great extent depends on the change in refractive index of a fat as it is hydrogenated. In practice, a small sample is removed from the converter, filtered, and the refractive index determined by the use of a good refractometer held at constant temperature. By reference to a predetermined curve relating the refractive index to the iodine value, a rapid estimation of the iodine value may be made. One source of error in this method is that *trans* double bonds formed during the hydrogenation affect the refractive index but not the iodine value. However if the reference curve is determined from the data on a hydrogenation under similar conditions of temmperature and pressure, this error does not become serious. Also since there is a time lag between taking the sample and completing the measurement, the rate of hydrogenation must be estimated if it is desired to stop at a certain iodine value.

Another method which may be used to give a quick estimation of iodine value is by measuring the dielectric constant of the oil. According to a recent patent (3) the permittivity, which is directly related to the dielectric constant, of an oil and its iodine value have a linear relationship over the commercially important range. Also the change in permittivity for a given change in degree of unsaturation is nearly constant for a large number of oils. The presence of catalyst in the oil does not affect the relationship as long as the catalyst concentration is constant. Therefore it is possible to set up a device that gives a continuous measure of the iodine value of an oil as it is being hydrogenated by circulating the oil from the converter through a conductance cell, where a continuous measure of the dielectric constant is made. A recorder and controller may be part of the apparatus, and by setting the controller for the desired change in dielectric constant, the hydrogenation may be stopped automatically at the desired change in iodine value. Although the apparatus required is quite extensive, this method would seem to be preferable to the refractive index method because of the possibility of continuous readings.

A third possible method of quick estimation of degree of saturation during hydrogenation is by the use of the Beta ray H/C meter developed by the Standard Oil Company. This meter makes use of the absorption of beta rays from the radioactive decay of Strontium 90. Since this absorption is related to the electron density of the sample and hydrogen has a greater number of electrons per gram than any other element, the instrument can measure the ratio of hydrogen to other elements. However the instrument is designed for use on hydrocarbons, and when applied to fats, its accuracy is limited to about 2.5 iodine value units.

The light absorption measurements, both infrared and ultraviolet are invaluable for the measurement

of special types of unsaturation, such as the *trans* configuration and conjugated systems. However these will be further discussed in other presentations. It is indeed fortunate that these methods are replacing the older chemical methods, such as the thiocyanogen number, Woburn iodine value, lead salt alcohol separation, diene value, etc., which were based on an empirical set of conditions and did not have too much fundamental basis.

Determination of the position occupied by the double bonds in unsaturated fatty acids has been the subject of a great deal of work. Oxidation of the unsaturated fatty acid at the double bond followed by isolation and identification of the oxidation products is one of the classical methods for determination of the double bond position. However the isolation and identification of all of the oxidation products is impossible by the usual methods of distillation or crystallization. By the use of chromatographic methods, which were recently developed and will be discussed in another presentation, the isolation, identification, and measure of all of the oxidation products becomes feasible. The method consists of oxidation of a small sample of the unsaturated fatty acid to a mixture of mono- and dibasic acids. Ozone is the preferred oxidizing agent because strong oxidizing agents, such as permanganate, degrade the acids during the oxidation. The samples of mixed acids are separated by a chromatographic system, and the percentage of each dibasic and monobasic acid is calculated as mole percentage. From this the position of the double bonds in the original sample may be estimated. For example, if a sample of monounsaturated acid of 18-carbon chain length is oxidized and only the 9-carbon dibasic acid, azelaic, is found, it is evident that all of the bonds were in the 9 position in the chain. However if the chromatographic analysis shows 10% of the 8-carbon dibasic, 80% of the 9-carbon dibasic, and 10% of the 10-carbon dibasic acids, the original sample was composed of 10% 8-octadecenoic, 80% 9-octadecenoic, and 10% 10-octadecenoic acids. Thus the positional isomers present in a mixture of monounsaturated acids may be determined by an analysis for the dibasic acids produced by oxidative cleavage. If there are two double bonds in the chain, their positions may be determined by an analysis for both mono- and dibasic acids. For example, a sample of linoleic acid, which has the double bonds at the 9 and 12 positions, would show on analysis only the 9-carbon dibasic and a 6-carbon monobasic acid. If a sample were composed of 50% 9–12 and 50% 9–11 octadecadienoic acids, it would show on analysis 100 mole percentage 9-carbon dibasic acid while the monobasic acid would be composed of 50% 6-carbon and 50% of the 7-carbon acids. From this it is evident that all of the samples had the first double bond at the 9 position, but 50% of the second double bonds were at the 12 position and 50% at the 11 position. The double bond positions in mixtures of mono- and dienes may also be determined. If a mixture of 50% 9-octadecenoic and 50% 9–12 octadecadienoic acids is analyzed, the analysis would show 100 mole percentage 9-carbon dibasic and 50% 6-carbon and 50% 9-carbon monobasic acids. It can readily be seen that all of the unsaturated acids had one bond at the 9 position, but 50% had another bond at the 12 position. The mixtures, such as encountered in studies of the hydrogenation of linoleic acid, are quite complex, but good estimates of the positional isomers may be made by this method.

To illustrate, the equations used in the calculations are shown as follows. The numbers in parentheses refer to double bond positions.

mole percentage 9-carbon dibasic = (9)+(9, 11)+(9, 12)
mole percentage 10-carbon dibasic = (10)+(10, 12)
mole percentage 11-carbon dibasic = (11)
mole percentage 12-carbon dibasic = (12)
mole percentage 9-carbon monobasic = ( 9)
mole percentage 8-carbon monobasic = (10)+(8, 10)
mole percentage 7-carbon monobasic = (11)+(9, 11)+(8, 11)
mole percentage 6-carbon monobasic = (12)+(8, 12)+(9, 12)+(10, 12)

By solving this series of equations, the amount of each isomer may be determined. An example of the analysis applied to partially hydrogenated linoleic asid is shown below.

ANALYTICAL RESULTS

| Monobasic acids | | Dibasic acids | |
|---|---|---|---|
| Chain length | Mole percentage | Chain length | Mole percentage |
| 6 | 61.7 | 9 | 58.8 |
| 7 | 12.2 | 10 | 14.9 |
| 8 | 13.2 | 11 | 12.8 |
| 9 | 12.5 | 12 | 13.5 |

61.7 = 6 mono = (9, 12)+(10, 12)+(12)
12.2 = 7 mono = (9, 11)+(11)
13.2 = 8 mono = (10)
12.5 = 9 mono = (9)

58.8 = 9 di = (9, 12)+(9, 11)+(9)
14.9 = 10 di = (10, 12)+(10)
12.8 = 11 di = (11)
13.5 = 12 di = (12)

RESULTS FROM CHROMATOGRAPHIC ANALYSIS OF ACIDS

| Octadecenoic acids | Octadecadienoic acids |
|---|---|
| ( 9) = 12.5% | ( 9, 12) = 46.3% |
| (10) = 13.2% | ( 9, 11) = 0 % |
| (11) = 12.8% | (10, 12) = 1.9% |
| (12) = 13.5% | total conjugated = 1.9% |
| total = 52.0% | total nonconjugated = 46.3% |

From analysis by ultraviolet method:
octadecenoic acids = 50.0%
conjugated dienes = 3.2%
nonconjugated dienes = 46.8%

There is still much work needed in the area of the determination of unsaturation of fats and derivatives. Of course, what we all would like would be a simple, accurate, and rapid method that would determine unsaturation of any type. However until better methods are developed, those described will suffice, provided their limitations are recognized.

REFERENCES

1. Benham, G. H., and Klee, L., J. Am. Oil Chemists' Soc., *27*, 127 (1950).
2. Boeseken, J., and Gelber, E. T., Rec. Tran. Chim., *46*, 158 (1927).
3. Goldsmith, K., Heselgrave, P. W., and Worstall, V. D., Brit. Patent 708,940 (May 12, 1954).
4. Hanus, J., Z. Untersuch. Nahr. Genussm., *4*, 913 (1901).
5. Kaufmann, H. P., Z. Unters. Lebensmittel, *51*, 3 (1926).
6. Norris, F. A., and Buswell, R. J., Anal. Chem., *15*, 258 (1943).
7. Ogg, C. L., and Cooper, F. J., Anal. Chem., *21*, 1400 (1949).
8. Pack, F. C., and Planck, R. W., J. Am. Chemists' Soc., *30*, 461 (1953).
9. Planck, R. W., Pack, F. C., and Goldblatt, L. A., J. Am. Oil Chemists' Soc., *30*, 417 (1953).
10. Rosenmund, K. W., and Kuhnhenn, W., Ber., *56*, 1262 (1923).
11. Von Hübl, A., J. Soc. Chem. Ind., *3*, 641 (1884).
12. Wijs, J. J. A., Analyst, *54*, 12 (1929).
13. Wijs, J. J. A., Ber., *31*, 750 (1898).