[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, UNIVERSITY OF BUFFALO MEDICAL SCHOOL]

# A Micro Method for the Determination of Iodine Numbers

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## Introduction

The basis of a method for the determination of the unsaturation values of lipid fractions containing sterols was briefly outlined in a previous paper.[1] This paper reports the application of a procedure, based upon those suggestions, to substances of differing structures.

The method requires no unusual apparatus, is simple, and permits the determination of both the total halogen consumed and the halogen acid produced, using a single small sample (0.75–25 mg.) of material.

In keeping with the findings of previous workers[1,2,3,4] carbon tetrachloride is used as the solvent for both the material and the reagent. The temperature of 0° is used in order to minimize substitution and oxidation reactions[1,4] and "thermal cleavage" of halogen acid.[4] Iodine monobromide, rather than bromine, is used as the reagent because of the greater stability of its carbon tetrachloride solution.[5] The usual custom of employing rather large quantities of potassium iodide in the titration of halogenating mixtures is not followed because a slight excess of potassium iodide over the bromine present is sufficient.

To secure accurate and duplicable results, the initial concentration of halogen in the reaction mixture should not be less than 0.05 milliequivalent per ml. and best results are obtained if the final concentration is about 80% of the initial quantity.

### Reagents

The reagents used are: *iodine monobromide*, 0.1 *N* in carbon tetrachloride; *potassium iodide*, 0.05 *N* in distilled water; *potassium iodate*, 0.5 *N* in distilled water; *starch*, 0.5% solution of potato starch containing no preservative; *sodium thiosulfate*, about a 0.07 *N* neutral solution. The last must be accurately standardized and must contain no carbon dioxide.

### Apparatus

Two micro burets of 5 ml., in 1/100 ml., capacity are recommended (macro burets may be substituted if thiosulfate of one-fourth the above concentration is used). A 2 ml. and a 6 ml. calibrated volumetric pipet. Three minutes for drainage is necessary for accurate delivery of carbon tetrachloride solutions. Two ordinary 1 ml. and one 5 ml. pipets. A T-shaped vessel, made from two 200 × 32 mm. lipless Pyrex test-tubes, is used for time-reaction measurements, or several determinations on aliquots taken from a quantity of reaction mixture (Procedure A). Iodine flasks and "shell" vials, 13 × 60 mm., are used for single observations or for cases in which much halogen acid is formed or insoluble products separate (Procedure B).

### Procedure

A weighed amount of the substance to be analyzed is dissolved in carbon tetrachloride and the solution is diluted so that 2 ml. contains enough material to react with an amount of halogen equivalent to one and one-half ml. of the 0.07 *N* thiosulfate.

(A) If *n* aliquot samples are to be titrated, $(n + 1) \times 2$ ml. of the above solution is run into the upright arm of the "T" vessel and $(n + 1) \times 4$ ml. of the halogenating reagent is measured into the side arm without allowing any to get into the vertical arm. The vessel is tightly stoppered with a *good cork* and placed in a bath at 0–1°. At the end of ten minutes the liquids are mixed and the vessel is replaced in the cold bath so that all the mixture is in the upright arm.

At the desired intervals, aliquot portions are withdrawn by means of the 6-ml. pipet and run into 5 ml. of 0.05 *N* potassium iodide contained in a 200 × 25 mm. test-tube. The mixture is then titrated with 0.07 *N* thiosulfate. This can be done to within 0.01 ml. The starch end-point is accentuated by partially enclosing the tube in a white towel to exclude transmitted light.

When the titration of the excess halogen is completed, 1 ml. of 0.5 *N* potassium iodate is added and the liberated iodine titrated with the thiosulfate. The figure is equivalent to the halogen acid[6] formed during the reaction, provided that the substance under examination is not too acidic[4] and that other complicating reactions have not occurred.[4]

## Calculations

Since the reagents are measured at room temperature and the samples are removed at 0° for titration, a factor accounting for the contraction of the fluid volume must be employed. It was found experimentally to be 0.987.

$$\text{Total Halogen Consumed} = [(B - T) \times 0.987 \times N \times 126.92]/W \times 100$$

expressed in terms of iodine number, where $B$ = blank titration at 0°, $T$ = back titration of the determination, $N$ = normality of the thiosulfate, 126.92 = milligrams of iodine equivalent to 1 ml. of 1 *N* thiosulfate, and $W$ = weight, in milligrams, of the material in the aliquot titrated.

(1) Ralls, THIS JOURNAL, **55**, 2083 (1933).
(2) Marshall, *J. Soc. Chem. Ind.*, **19**, 213 (1900).
(3) Böeseken and Gelber, *Rec. trav. chim.*, **46**, 158 (1927).
(4) Buckwalter and Wagner, THIS JOURNAL, **52**, 5241 (1930).
(5) Yost, Anderson and Skoog, *ibid.*, **55**, 552 (1933).
(6) Schweitzer and Lungwitz, *J. Soc. Chem. Ind.*, **14**, 130 (1895).

If the blanks are run at room temperature, the calculation would be according to the formula

$$[(B - T \times 0.987) \times N \times 126.92]/W \times 100$$

$$\text{Halogen Acid} = [T_{HX} \times 0.987 \times N \times 126.92]/W \times 100$$

expressed as iodine number, where $T_{HX}$ is the number of ml. of thiosulfate required to reduce the iodine liberated upon the addition of the potassium iodate.

Bound or Organic Halogen = Total Halogen Consumed − Halogen Acid

(B) Exactly 2 ml. of the carbon tetrachloride solution of the substance to be investigated is placed in an iodine flask into which is then inserted an open "shell" vial containing exactly 4 ml. of iodine monobromide. The flask is tightly stoppered, sealed with a few drops of 0.05 *N* potassium iodide or iodide and placed in a bath at 0°. After about ten minutes the flask is carefully tilted to allow the solutions to mix and is then replaced in the bath. The trough around the stopper is filled with 5 ml. of the iodide solution. At the end of the appropriate time (forty to sixty minutes is ordinarily required for complete saturation), the vessel is partially opened to allow the iodide to run down the inside wall, then closed and gently shaken. Now the stopper is removed and carefully washed.

The excess halogen and the halogen acid are titrated. The calculations are like those of Procedure A except that the volume correction factor, 0.987, is *not* used.

(C) The directions given for B may be modified for use with much smaller quantities in the following manner: (1) 2 ml. of carbon tetrachloride containing 1.5 to 10 mg. of material is treated with 2 ml. of the regular halogen reagent and all titrations are made with 0.035 *N* thiosulfate; or (2) 1 ml. containing 0.75 to 5 mg. of substance is treated with 1 ml. of iodine monobromide. The titrations require 0.0175 *N* thiosulfate. In these modifications, micro burets are *necessary* and smaller vials (10 × 40 mm.) are more convenient for the transfer of the iodine monobromide to the flasks.

## Results

All the results reported were obtained by means of Procedure A or B; however, the modifications gave the same results as A in those cases where it was tried, cholesterol, oleic acid and octylene.

The curves given in Fig. 1 are typical of all the substances which showed no abnormalities. In the following list are given, respectively, the name of the substance, the quantity used, the value found by Procedure A, and the theoretical or commonly accepted iodine number.

Corn oil, 9.56 mg., 123.5, 111–128.
Cotton seed oil, 11.61 mg., 109.3, 109.
Cod liver oil, 9.04 mg., 164, 164–170.
Coconut oil, 8.21 mg., 5.0, 5–10
Oleic acid, 14.34 mg., 90.0, 89.9.
Ethyl oleate, 12.12 mg., 85.5, 84.5.
Brucic acid, 13.50 mg., 74.0, 75.1.
Lubricating oil, Socony No. 28, 10.00 mg., 36, 18 (company's unsaturation value).
Undecylenic acid, 8.60 mg., 135.0, 137.0.
Octylene, 5.63 mg., 224, 226.
β-Cholestanol, 15.00 mg., 0.0, 0.0.
Cholesterol, 15.00 mg., 66.0, 65.7.
Cholesteryl acetate, 16.70 mg., 59.5, 59.2.
Cholesteryl benzoate, 11.10 mg., 51.8, 51.8.
Triphenylmethylcholesteryl ether, 24.40 mg., 40.5, 40.4.

None of the above substances reacted to produce halogen acid, with the exception of the lubricating oil which gave a halogen acid value of 8.4.

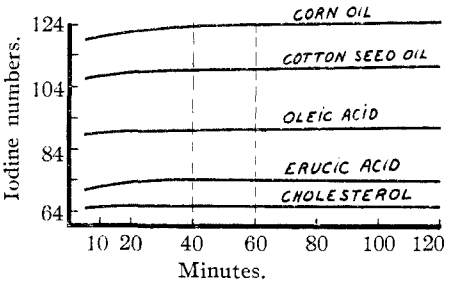


Fig. 1.—The effect of time of reaction upon the iodine numbers of certain substances studied according to Procedure A.

Not all substances yielded good values with the procedures described (Table I). Castor oil produced so much halogen acid that $HI_3$ or $HBr_3$, or both, separated out and caused much trouble in Procedure A. The separated dibromoricinoleic acid caused some difficulty too. It was hard to obtain any values at all with crotonaldehyde because that substance displayed a marked reversible cleavage of halogen.[2,4] Ergosterol showed the anomalies described by Copping[7] and this substance is being subjected to further study.

Study of the results given shows that we cannot put forth the described method as the "universal" method in which all reactions, save addition, are completely prevented in all cases. Nor do we feel that the custom of subtracting twice the halogen acid from the total consumed halogen and calling the result the halogen of addition is fully justified—because substitution is not the only side reaction producing halogen acid.

(7) Copping, *Biochem. J.*, **22**, 1142 (1928).

TABLE I

THE RELATION BETWEEN THE TIME OF REACTION AND THE IODINE NUMBER OF SUBSTANCES SHOWING SOME ABNORMALITY

The lower figure in each column after the name of the substance represents the halogen acid for that substance. Blank spaces indicate 0.0 halogen acid, while ... represents no determination.

| Substance | Quantity, mg. | Duration of reaction in minutes 10 | 20 | 40 | 60 | 90 | 120 | 150 | 180 | Calcd. |
|---|---|---|---|---|---|---|---|---|---|---|
| By Procedure A | | | | | | | | | | |
| Castor oil | 5.55 | 92.0 | 97.0 | 109.0 | 125.0 | 145.5 | 154.0 | 160.0 | 165.3 | 86.0 |
| | | 7.5 | 8.5 | 9.0 | 7.5 | 4.5 | 2.0 | 1.2 | 0.3 | 0.0 |
| Cyclohexane | 4.31 | 320.0 | 320.0 | 320.0 | 320.0 | 320.0 | 320.0 | ... | ... | 305.0 |
| | | | | | | | | ... | ... | 0.0 |
| *d*-Limonene | 5.60 | 351.0 | 354.0 | 358.0 | 361.0 | 366.0 | 371.0 | ... | ... | 373.0 |
| | | 8.7 | 9.0 | 9.1 | 9.3 | 9.5 | 10.0 | ... | ... | 0.0 |
| Ethyl maleate | 9.08 | 1.0 | 2.0 | 3.0 | 4.0 | 6.0 | 7.2 | 8.4 | 9.4 | 147.0 |
| | | | | | | | | | | 0.0 |
| Crotonic acid | 3.44 | 1.0 | 3.5 | 8.0 | 12.0 | 20.0 | ... | ... | ... | 295.0 |
| | | | | | | | ... | ... | ... | 0.0 |
| Cinnamic acid | 5.92 | 5.0 | 9.0 | 15.0 | 19.0 | 26.0 | 36.5 | 39.2 | ... | 171.4 |
| | | | | | | | | | ... | 0.0 |
| Mesityl oxide | 4.02 | 163.0 | 186.0 | 204.0 | 213.0 | 225.0 | 242.0 | 251.0 | 256.0 | 259.0 |
| | | 15.0 | 16.0 | 17.0 | 17.0 | 17.0 | 17.0 | 17.0 | 17.0 | 0.0 |
| Crotonaldehyde | 3.10 | 309.0 | 311.5 | 312.0 | 314.0 | ... | ... | ... | ... | 362.0 |
| | | [a] | ... | ... | ... | ... | ... | ... | ... | 0.0 |
| Phenanthrene | 9.15 | 5.0 | 8.5 | 13.2 | 17.0 | 22.6 | 27.5 | 32.0 | 36.3 | 142.6 |
| | | 0.5 | 1.0 | 2.0 | 2.8 | 4.0 | 5.0 | 6.2 | 7.1 | 0.0 |
| By Procedure B | | | | | | | | | | |
| Castor oil | 5.55 | 80.0 | 83.5 | 87.0 | 90.0 | 93.5 | 94.5 | 95.2 | 96.0 | 86.0 |
| | | 7.5 | 8.5 | 10.5 | 11.2 | 12.0 | 12.0 | 12.0 | 12.0 | 0.0 |
| Pinene | 5.95 | 216.0 | 227.0 | 241.5 | 252.0 | 265.0 | 274.0 | 280.0 | 284.0 | 186.2 |
| | | 15.2 | 16.2 | 16.5 | 16.5 | 16.5 | 16.5 | 16.5 | 16.5 | 0.0 |
| Retene | 12.00 | 33.5 | 40.5 | 48.0 | 53.6 | 59.5 | 64.1 | 64.6 | 72.8 | 108.4 |
| | | 6.6 | 12.0 | 18.5 | 19.6 | 21.5 | 23.5 | 25.7 | 27.5 | 0.0 |
| Ergosterol | 5.28 | 283.0 | 312.2 | 336.4 | 348.0 | 354.0 | 359.0 | 362.0 | 365.0 | 192.1 |
| | | 114.4 | 134.0 | 150.5 | 156.0 | 160.2 | 164.0 | 166.8 | 169.7 | 0.0 |
| Ergosteryl benzoate | 6.67 | 265.0 | 279.0 | 295.0 | 304.0 | 311.3 | 318.0 | 321.6 | 325.5 | 152.0 |
| | | 134.2 | 142.1 | 154.6 | 161.5 | 166.2 | 171.1 | 173.5 | 176.2 | 0.0 |

[a] The reversible cleavage of halogen was so pronounced that no determination of halogen acid could be made.

## Summary

A method is described which is applicable to samples of from 0.75 to 25.00 mg. and which permits the determination of the total halogen consumed and the halogen acid produced, using a single sample.

The results are given for thirty compounds.

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