THE DETERMINATION OF IODINE IN BLOOD, FOODS, AND URINE*

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(Received for publication, July 23, 1932)

Iodine estimations in which organic matter is destroyed by open combustion have proved very discouraging. Orr (1) investigating the iodine methods suitable for the analyses of foods, soil, blood, etc., and their application to the study of goiter concludes, "It has been made clear that the present methods in use for the estimation of iodine in very minute quantities and in the presence of organic materials are so unsatisfactory as to impede further progress in research. There is need for a standard method of estimation suitable for use by all workers alike that will give results closely approximating to accuracy, or results in which the standard error is constant and known."

Four iodine determinations on a vegetable preparation after combustion in a muffle furnace at 400–450° gave the following results: 0.17, 0.16, 0.23, and 0.25 mg. per kilo. Through the courtesy of the South Carolina Food Research Commission four determinations on a similar preparation were made for us with equally great variation. We have never been able to run satisfactory duplicates even at a temperature as low as 350°. When iodides are added to foods, losses of 10 to 25 per cent or more occur during incineration; the results are irregular and uncontrollable. We find that even in the presence of weak alkali, iodides are lost when heated at comparatively low temperatures.

We therefore turned to the use of a closed system with an absorption train. All the methods of this class that have been described are elaborate, time-consuming, and not very satisfactory. In theory at least the McClendon method (2) in which a Cottrell

* Presented before the American Society of Biological Chemists at Philadelphia, April 28–30, 1932 (J. Biol. Chem., 97, p. xc (1932)).
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precipitator is used to condense the smoke cloud arising from combustion should be capable of yielding good analyses but the work of assembling the apparatus, of carrying out the combustion, and then of washing out the apparatus with its long absorption train, is so great that not more than one or two combustions can be made in a day. Since McClendon's original paper was published, he has abandoned this procedure in cases in which the amount of iodine present is relatively large and has advocated the use of a muffle furnace for incineration instead of the large combustion tube and Cottrell precipitator (3).

In view of all this we hoped that better results might be obtained by making the combustion in a bomb. For this purpose an Emerson bomb proved fairly satisfactory. About six combustions could be made in a day but the maximum charge could be only a little over a gm. This serious limitation was overcome by burning the substance in a flask in a current of oxygen. We have used this type of apparatus for more than a year. Such a combustion proceeds quietly and comparatively quickly but it gives rise to a smoke cloud which cannot be absorbed by passing through half a dozen or more efficient gas wash bottles. A simple device described by Weber (4), consisting of two funnels placed mouth to mouth with some filter papers between and made gas-tight with paraffin, proved remarkably efficient. With this simple apparatus we have been able to obtain consistent results, using the methods of Leitch and Henderson (5) and of Kelly and Husband (6) for the actual estimation of iodine, somewhat modified to meet our conditions. The accuracy possible is limited by the exactness with which one can measure 0.05 cc. and with the pipette used here, this is about 10 per cent. When 0.002 to 0.01 mg. of iodine is present, the accuracy of the method is 3 to 5 per cent.

The value of this apparatus for incineration of biological material in the estimation of minute amounts of inorganic constituents, and especially of the more volatile ones, will be apparent.

Reagents

1. Distilled Water - Add enough potassium permanganate to distilled water to give a purple color and let it stand 24 hours. To 8 liters of such water add about 30 cc. of 50 per cent sodium hydroxide, a little charcoal to prevent bumping, and distil, using
either glass or rubber stoppers for connections and a glass or tin condenser.

2. Potassium Hydroxide—A 10 per cent solution of reagent grade kept in a paraffin coated bottle.

3. Hydrogen Peroxide—30 per cent Merck's superoxol is distilled in vacuo at a temperature below 60°. The peroxide is not allowed to come in contact with organic matter but rubber stoppers are used on the distillation flasks. In an ice box this reagent will keep for months with little decomposition.

4. Bromine—Bromine is purified by shaking 50 cc. in a separatory funnel five times with 10 cc. of 0.1 N NaOH. It is then distilled under a little very dilute alkali in an all-glass distilling apparatus or from a retort that fits loosely into a condenser without any stopper.

5. Sulfuric Acid—3 and 20 per cent solutions.

6. Sodium Bisulfite—10 per cent solution kept in an ice box. It keeps for at least 2 weeks.

7. Potassium Iodide—A 30 per cent solution made up the day it is to be used. We have found several of the analyzed grades to yield molecular iodine in solution much more readily than the U.S.P. grade.

8. Soluble Starch—A 1 per cent solution.

9. Methyl Orange—A 0.01 per cent solution.

10. Pieces of anthracite coal about 1 to 2 mm. in diameter, which are used to prevent bumping and for the rapid removal of bromine from solution (7). These pieces are cleaned by boiling in 10 per cent nitric acid, washing free of acid, and then heating to glowing in a platinum or silica dish.

11. Sodium thiosulfate solution, 0.001 N, standardized against potassium iodate solution as follows: 2 cc. of 0.001 N potassium iodate solution are measured with a calibrated pipette into a 50 cc. conical flask. Add 8 cc. of water, 0.6 cc. of 3 per cent sulfuric acid, and 0.5 cc. of 30 per cent potassium iodide. Titrate until the yellow color becomes faint, add 0.25 cc. of starch solution, and continue titration until the blue color disappears. A 2 cc. micro burette graduated in 0.01 cc. and delivering a drop not larger than 0.02 cc. is used. In an ice box this solution will not change significantly for at least 2 weeks.
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Apparatus

Fig. 1 shows the system used. It consists of a 3 or 5 liter balloon flask, W, fitted with a rubber stopper through which enter (1) a Pyrex glass tube, e, 5 to 6 mm. inside diameter, flared at the bottom, through which oxygen passes into the flask, (2) an outlet tube, G, and (3) two chromium steel rods, d and d', about 3.5 to 4.0 mm. in diameter. One of these rods is bent at the bottom into a ring and serves as a holder for the vessel in which the charge is burned as well as for one of the terminals for conducting a small electric current. The charge is placed in a porcelain crucible or iron dish of suitable size and a platinum wire, 0.025 mm. in diameter and 3 to 4 cm. long, connects the terminals and is brought in contact with the charge. For 10 cc. of dried blood a wide mouthed crucible with a top diameter of 35 mm. is used. The clamp furnishes a convenient means of fastening the rubber stopper.

Fig. 1. Set-up of the apparatus used for iodine determinations. W is the balloon flask fitted with a rubber stopper through which enter Pyrex glass tube, e, two chromium steel rods, d and d', and an outlet tube, G. X and Y are ordinary Drexel wash bottles. Z consists of two funnels placed mouth to mouth so as to be gas-tight, which serve as a smoke condenser.

1 Constructed by Mr. H. Hawker, mechanic to the laboratory.
into the combustion flask but is not essential; wire may be used instead. The clamp consists of a metal collar around the flask and a metal plate on the rubber stopper, through which the tubes and rods pass with generous openings, so that the terminals, \( d \) and \( d' \), do not come into contact with the plate. It is fastened to the collar by means of two thumbscrews. The ordinary Drexel wash bottles, \( X \) and \( Y \), are 250 cc. in size and the smoke condenser, \( Z \), consists of two 6.5 cm. funnels placed mouth to mouth with four pieces of closely woven analytical filter paper between them (Schleicher and Schüll No. 589, blue ribbon, or Whatman No. 42, etc.).\(^2\) The funnels are held firmly in a suitably constructed clamp or frame and made gas-tight by coating the edges with clean paraffin of high melting point. The parts of the set-up are connected as closely as possible with gum tubing.

An exceptional degree of cleanliness is necessary. Apparatus should be washed with cleaning solution, rinsed with distilled water, and finally with the doubly distilled water.

To insure good contact, the iron electrodes with the platinum wire are dipped into boiling 10 per cent hydrochloric acid for a moment and rinsed with water as above.

**Preparation of Sample for Analysis**

*Blood*—10 cc. or more are pipetted into small flat evaporating dishes and dried in an oven at 80–85° for 6 hours. The dried mass is broken up by hand and the part that adheres to the dish is scraped off with a sharp edged steel spatula and all brushed into a porcelain crucible.

Food is hashed and similarly dried at 80–85°.

Urine is dried on a water bath with enough caustic alkali to make it strongly alkaline. Substances containing comparatively little organic matter, like urine, will not burn easily. They are

\(^2\) Washing a few hundred cc. of water through the best grades of filter paper gives a filtrate that contains a considerable amount of organic matter. We have therefore found it necessary to wash the best paper obtainable with a large volume of 0.01 per cent KOH several times, then four times with distilled water, and once or twice with doubly distilled water. The paper is sucked nearly dry on a Buchner funnel and dried in an oven at 80–90°. This paper is used for the smoke absorber. Filtrations are all made through glass Buchner funnels with fused-in discs of sintered glass of the finest porosity.
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therefore mixed with a little iodine-free dry powdered sugar, salicylic acid, or other material rich in carbon.

Analysis

A. Oxidation—In the wash bottles X and Y place 150 cc. and 100 cc. of water respectively. When working with 0.001 to 0.004 mg. of iodine, 0.05 cc. of 10 per cent potassium hydroxide is added to Y; with 0.005 to 0.03 mg. of iodine, 0.4 cc. of 10 per cent potash is used. The crucible containing the charge is set into the chromium steel ring, the platinum ignition wire being adjusted so that good contact is made with the charge. The rubber stopper (with its contained parts) is set carefully into the flask and fastened; and the flask, wash bottles, and smoke condenser connected as shown in Fig. 1. Pass oxygen through the apparatus for a minute or so at a rate of about 600 cc. per minute, avoiding sudden changes in the rate that might break the paraffin seal of the smoke condenser. Then ignite the charge; the material bursts into flame. Continue passing oxygen through until the charge is burned up and the white fumes remaining in the flask have been sent through the smoke filter. Disconnect the chain beginning at the distal end.

B. Preparation for Analysis—Cut the smoke condenser apart with scissors and drop the filter papers into an 800 cc. beaker; rinse the funnels into it. Place the crucible in the same beaker, rub the sides well with a policeman, wash the stopper and its tubes and rods into the beaker, and finally pour the contents of the wash bottles into the same beaker and wash these also. The washings, which should be about 450 cc. or less, are heated to about 40° and filtered through a 45 mm. glass Buchner funnel with disc of sintered glass of finest porosity and washed into a 1 liter evaporating dish. At this point add 0.35 cc. of 10 per cent potassium hydroxide when working with 0.001 to 0.004 mg. of iodine (with 0.005 to 0.03 mg. of iodine use 0.4 cc. of potassium hydroxide) and then 5 cc. of 30 per cent hydrogen peroxide or more depending on the yellowness of the filtrate. Evaporate on a water bath to 3 to 4 cc., filter off the iron hydroxide, etc., through a 30 mm. glass Buch-

3 This is a convenient point to interrupt the analysis, if necessary, as the peroxide can carry on its action in the interval. A glass-stoppered conical flask is a good vessel for storing the solution.
ner funnel, and collect filtrate and washings in a 100 cc. beaker. Concentrate on a water bath or hot plate to 1 to 2 cc., transfer to a 25 cc. platinum dish, and concentrate to a few cc. Add 0.05 cc. of 10 per cent potassium hydroxide (with 0.005 to 0.03 mg. of iodine use 0.7 cc.) and continue evaporating just to dryness. Place in a vacuum desiccator overnight.

In the analysis of urine or of foods where 10 gm. or more of material are used, it is necessary to extract at this point with about 8 cc. of 80 per cent ethanol. (This is purified by distilling from alkali.) The salts are filtered off and washed five or six times with 2 cc. portions of 80 per cent alcohol. The filtrate and washings are evaporated just to dryness in a 25 cc. platinum dish.

A small amount of organic matter is still present, which must be removed completely before the determination may be made. This has usually been accomplished by incinerating, Stolte fashion, for a minute or less or until the material just fuses. Large losses not infrequently occur at this point. We have, therefore, preferred to carry out the final combustion in an inclosed system (Fig. 2) and for this purpose have used a wide mouthed, 1 liter conical flask fitted with a rubber stopper through which pass (1) an entry tube reaching to about 4 cm. from the bottom and an exit tube, both of which are 5 to 6 mm. in diameter, (2) two chromium steel rods 2.5 mm. in diameter, which act as terminals; one of these is bent into a circle at the bottom and serves, with a short cross strip of iron, as a support for a heating unit and the platinum dish. The heating unit is made from 4 feet of 0.025 mm. platinum wire wound into a coil about 1.5 mm. in diameter, which is arranged in a spiral and fastened at many points on a 4 cm. perforated porcelain filtering plate. The heating unit is placed on the iron ring and the ends of the coil fastened to the terminals; another perforated porcelain plate (from the bottom of a Gooch crucible) covers the coil and on this the platinum dish is set, avoiding direct contact with the terminals or heating coil. The rubber stopper with its fittings is then set in the conical liter flask, connected to a very small gas wash bottle containing 15 to 20 cc. of water. Oxygen is passed through for a few seconds. Connect the terminals with a rheostat, and slowly increase the current going through the coil so that sputtering is avoided, until the coil glows quite brightly. Continue heating for 10 to 15 minutes. Turn off the current,
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disconnect the apparatus, and cool the flask in running water. Wash into a 250 cc. beaker and evaporate on a hot-plate or water bath to 1 to 2 cc., shaking if necessary to prevent drying in spots. When 0.001 to 0.004 mg. of iodine is present, filter through a small glass filter, transfer to a 30 cc. conical flask, and reduce the volume to 7 to 8 cc., while with 0.005 to 0.03 mg. of iodine transfer to a 50 cc. flask, concentrating the volume to 15 cc.

Fig. 2. The inclosed system used for the final combustion consists of a 1 liter flask fitted with a rubber stopper through which pass an entry tube and two chromium steel rods which act as terminals, one of which serves as a support for the heating unit and platinum dish.

C. Determination of 0.001 to 0.004 Mg. of Iodine—Add a drop of methyl orange solution and just acidify with 3 per cent sulfuric acid (about 0.3 cc. is needed); add 0.7 cc. of 3 per cent sulfuric acid, 0.4 cc. of 10 per cent sodium bisulfite, and a piece of washed coal.

The solution may be very slightly yellow due to the presence of a little iron; this does no harm. If, however, the color is due to organic matter, indefinite end-points are obtained and the results will be high.
Boil for exactly 3 minutes and cool at once. Add 2 or 3 small drops of bromine and shake until the fluid is orange-yellow. Boil off the bromine and continue concentration until the volume is reduced to 2.5 cc. ± 0.5 cc., cool, and remove the coal with a pair of slender forceps. Add 0.2 cc. of (freshly prepared) 30 per cent potassium iodide and 2 drops of a 1 per cent soluble starch solution, and titrate immediately with 0.001 N thiosulfate with a 0.2 cc. pipette graduated in 0.01 cc. according to Leitch and Henderson.

D. Determination of 0.005 to 0.03 Mg. of Iodine—Add a drop of methyl orange and acidify with 20 per cent sulfuric acid; add 0.8 cc. of 20 per cent sulfuric acid, 0.4 cc. of sodium acid sulfite, and a piece of coal. Boil for exactly 2 minutes, cool at once, add 2 or 3 small drops of bromine, and shake until the solution is a deep orange-yellow. Boil until the bromine color has disappeared and then for exactly 2 minutes more. Add a small pinch of salicylic acid (10 mg.), cool, and remove the coal with forceps at once. Add 0.5 cc. of 30 per cent freshly prepared potassium iodide and 0.25 cc. of starch solution and titrate at once with 0.001 N thiosulfate with a 2 cc. micro burette graduated in 0.01 cc. and delivering a drop not larger than 0.02 cc.

Notes on Method—Each step of the Kelly and Husband and the Leitch and Henderson methods that we have used has been thoroughly tested and adapted to our conditions. The results of this examination as well as other findings will be discussed in these notes.

1. Alkali iodides are more volatile than has usually been supposed. Significant losses occur when they are heated in an oven at 85°. For example, portions of potassium iodide equivalent to 0.0102 mg. of iodine were heated in conical flasks in an oven at 85° for 24 hours. The iodine was then estimated with the following recoveries: 0.0093, 0.0087, 0.0077, 0.0089 mg. When heated under similar conditions or on the water bath for half an hour, with 0.1 gm. of potassium hydroxide, no iodine was lost. Even when heated in a crucible for 2 minutes with 0.1 gm. of potassium hydroxide, according to Stolte, no loss of iodine occurred starting with 0.001 mg. or 0.01 mg. of iodine. Since the hydroxide added could easily be converted to carbonate in the course of the analysis, we made similar tests, with sodium car-
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bonate in place of potassium hydroxide. Heated just to dryess on the water bath with 0.1 gm. of sodium carbonate, only 0.0090 mg. of iodine was recovered from 0.0102 mg. When the heating on the bath was continued for half an hour, only 0.0076 mg. of iodine was recovered.

From figures such as the above, which have been obtained many times, we may conclude that heating iodides alone or with carbonate at temperatures below 100° for a comparatively short time results in serious losses of iodine while in the presence of caustic alkali such treatment does not result in loss of iodine.

2. Because the halides are converted largely to chlorates, bromates, and iodates during the combustion, similar tests were made with potassium iodate. When heated in the oven at 85° or on the water bath, even without the addition of alkali, no iodine was lost.

3. Since chlorates and bromates must be reduced to the corresponding halide before the determination can be made, rather large amounts of sodium bisulfite are used. For this reason and because the composition of the bisulfite is apt to vary, a separate blank test is required each time new sulfuric acid or sulfite solution is made up so that the proper proportions of both can be used. Put 0.4 cc. of 10 per cent sodium bisulfite, 0.7 cc. of 3 per cent sulfuric acid, a piece of coal, and 5 cc. of water into a 30 cc. flask, boil for 3 minutes, cool, and add 2 drops of bromine. Shake until deep yellow; boil until the volume is reduced to 2.5 cc. Cool and add 0.2 cc. of 30 per cent potassium iodide and 2 drops of starch.

If a permanent yellow is not obtained rapidly on adding bromine and shaking, too much bisulfite is present. If after oxidation with bromine a blue color develops in less than 2 or 3 minutes after adding potassium iodide and starch, there is too much acid present.

4. With 0.001 to 0.004 mg. of iodine, the volume in which the final titration should be made must be between 2 and 3 cc. In this volume, the amount of 30 per cent potassium iodide may vary between 0.2 and 0.4 cc., while that of 3 per cent sulfuric acid may vary between 0.6 and 0.9 cc. We have used 0.2 cc. and 0.7 cc. respectively.

5. With 0.005 to 0.03 mg. of iodine, the titration should be made in a volume of 10 to 15 cc. In this volume the amount of 30 per cent potassium iodide and 20 per cent sulfuric acid may both vary
between 0.5 and 0.1 cc. We have used 0.5 cc. and 0.8 cc. respectively.

To test the reliability of the method, we have estimated the amount of iodine in pure iodides and iodates, with results that approach the theoretical as closely as our apparatus permits. In addition, amounts of potassium iodide varying from 0.001 to 0.01 mg. of iodine have been dried down with 10 cc. of mixed human blood and good recoveries of the added iodine have been obtained. Results of twelve such analyses are presented in Table I.

It must be emphasized that the estimation of iodine in blood, foods, etc., by any method requires a most exacting technique and that the danger of contamination is very great, scrupulous care being necessary to prevent it. It is necessarily an elaborate and delicate operation but we have found that in skilled hands a more rapid and controlled combustion can be obtained with this comparatively simple and easily assembled apparatus than by open combustion methods and a better economy of time and more accurate results are possible than by the closed combustion methods now in use.

Addendum—Since this paper was submitted for publication, Carns (8) has described a somewhat similar but much more elaborate incinerating apparatus in which the products of combustion are collected in an absorption train that includes a Cottrell precipitator.
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