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THE DETERMINATION OF TRACES OF IODINE. I.

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Since iodine is one of the rarest chemical substances and is absolutely essential to the life of human beings and other mammals, the determination of traces of this element is of some chemical importance. According to the atomic disintegration theory of the origin of the elements, the usual method of expulsion of a helium nucleus has led to the superabundance of the elements of even atomic weight. Those of uneven atomic weight are relatively rare and iodine is one of the rarest. Its wide distribution supports the idea that it was formed in place. It is not abundant enough to be acted on by the ordinary geological concentrating forces; there are no iodine mines and only small deposits of silver iodide.

If sufficient iodide is present for weighing, it may be precipitated as palladous iodide (PdI_2) by the addition of palladous chloride to the acid solution. After standing for two days in a warm place the precipitate will be coarse enough to filter through a Gooch crucible. It may then be washed with warm water, dried at 100° and weighed. Although Stieglitz[1] uses this reaction for microchemical detection of iodine in tissues, he does not use the balance; in fact, the sample is usually too small for gravimetric determination.

Iodide or iodine may be quantitatively oxidized to iodate by the action of bromine (as in Kendall's[2] method) or chlorine. The excess of bromine or chlorine may be boiled out. On the addition of an excess of potassium iodide, six times as much iodine is formed as the total of the element that was originally present in the sample. In the titration of iodine, starch is a sensitive indicator when iodide is also present. (Starch solution has reducing action only at elevated temperature.) The addition of iodide in the iodate method or to increase the sensitivity of the starch iodide reaction, necessitates the removal of all oxidizing agents which would liberate iodine from iodide. The oxygen of the air will liberate iodine from iodide, particularly in sunlight or in the presence of catalysts such as iron or other heavy metals. The heavy metals may be almost completely removed, even in acid solution, by the addition of phosphoric acid and filtration.

It may seem safer to analyze samples for the first time without the addition of iodide but, on the contrary, by the use of reagents which have

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[1] Stieglitz, *J. Pharmacol.*, **22**, 89 (1923).

[2] Kendall, *J. Biol. Chem.*, **43**, 149 (1920).

been carefully freed from iodine by recrystallization or distillation over alkali. Potassium salts and bromine are more likely to contain traces of iodine than sodium salts and chlorine. Nitric acid made from Chilean saltpeter is more liable to contain iodine than nitric acid made from the air. According to my own observations and those of Gautier, all the iodine in the atmosphere is in the form of dust.

In the oxidation of iodide to iodine, careful selection of the oxidizing agent and control of the conditions are necessary in order to prevent the oxidation from proceeding too far (formation of iodate). Iodide may be oxidized to iodine by permanganate or by the action of nitrous acid in slightly acid solution, with simultaneous production of nitric oxide. In a moderate concentration of hydrogen ions the formation of nitric acid would be very small and would be retarded by preventing the escape of nitric oxide. If oxygen is present, some of the nitric oxide will be reoxidized to nitrous acid. The production of nitrogen gas is very slow and may be neglected. The iodine may be determined by the extraction method introduced by Rabourdin in 1850. If a water solution of iodine is shaken with an equal volume of carbon tetrachloride, about $^{85}/_{86}$ of the iodine passes into the carbon tetrachloride. If the water is 10 times the volume of the carbon tetrachloride, about $^{85}/_{95}$ of the iodine passes into the latter. If sulfates or nitrates are added to the water, more of the iodine passes into the carbon tetrachloride but if chlorides are added to the water, less of the iodine passes into the carbon tetrachloride. This effect is still greater with bromides but fortunately they are never in very high concentration. Iodides do not interfere in this form of analysis because all of the iodide is oxidized to iodine and hence none is present as iodide in the water solution. It is very desirable to reduce the concentration of chlorides and this may be accomplished by evaporating the solution to dryness and extracting it with absolute alcohol, which dissolves the iodide but very little of the chloride.

In the presence of organic substances of high molecular weight, the above methods of analysis of iodine fail. Even drinking water or sea water may contain too much organic matter for iodine analysis. Organic matter is usually destroyed by ashing but ashing in open dishes is often attended by loss of iodine even in the presence of alkali. It is for this reason that the method given below has been elaborated.

Procedure

In a previous paper,[3] some parts of the procedure were given and some improvements were indicated in a later note,[4] whereas the theoretical considerations and literature were given in a review.[5] More experience has since been gained in the determina-

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[3] McClendon, *J. Biol. Chem.*, **60**, 289 (1924).

[4] McClendon, *Proc. Soc. Exptl. Biol. Med.*, **23**, 494 (1926).

[5] McClendon, *Physiol. Rev.*, **7**, 240 (1927).

tion of traces of iodine, the method somewhat improved and simplified, and iodine analyses made of drinking water, sewage, cereal grains, vegetables, fruits, milk, butter, fish, mammalian tissues, urine, feces and other substances. In the ashing, the samples were burned in a large silica combustion tube open at one end. To avoid loss due to air currents at the opening of the tube, an attempt was made to close it partially with a sheet iron door but this was later replaced by an ordinary tinned funnel which served as a door and also for admission of oxygen. When oils were burned in the tube, it was found desirable to spray them in continuously. An apparatus was made for injecting powdered samples of foodstuffs in a similar manner, but if the combustion tube was only moderately heated, some particles of dust escaped combustion and when it was heated to a high temperature, the dust adhered to the automatic feed and clogged it. An automatic stoker in the form of a moving grate was next tried but it was found desirable to remove the automatic mechanism and move it continuously by hand.

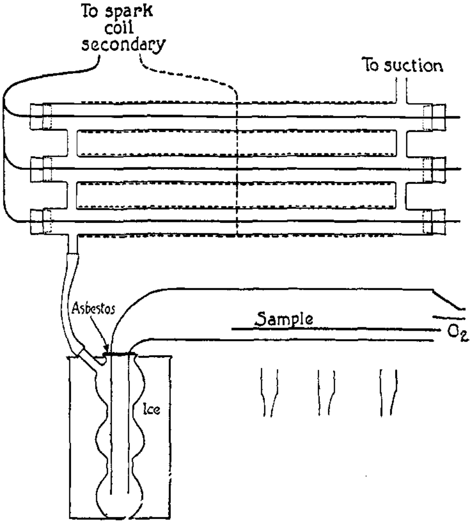

Fig. 1.

An atomizer for spraying in melted butter and an apparatus for feeding in milk powder will be described in a second paper.

The apparatus is shown in Fig. 1 and consists of a fused silica tube of 9 cm. bore and 60 cm. long with an elbow of 2.5 cm. bore and 30 cm. long, bent down into a Pyrex absorption flask containing 500 cc. of distilled water, to which 0.1 g. of sodium hydroxide has been added, and immersed in ice water. The neck of the flask is made air tight around the elbow by means of wet asbestos fibers. There is a side neck to the absorption flask leading to the Cottrell precipitator, which consists of 3 lead-glass tubes, 90 cm. long and 2.5 cm. bore, connected together in parallel by side necks (1.25 cm. bore) near each end. The tubes are closed with rubber stoppers through the centers of which run fine nichrome wires extending through the centers of the tubes and connected to one pole of the secondary of a Ford spark coil. The other pole of the spark coil is connected by means of a copper wire to tin foil covering the tubes. The spark coil is operated by a 6-volt storage battery and builds up a very high potential in one direction. By means of the side necks, one end of the Cottrell precipitor is connected with the absorption flask and the other end through 2 (or more) wash bottles (not shown in the figure) with a rotary suction pump. Each wash bottle contains 100 cc. of distilled water to which is added 0.02 g. of sodium hydroxide.

The open end of the silica tube is closed with a tinned funnel, admitting oxygen from a cylinder, and the lower edge of the funnel is bent up so that the sample may be introduced. The pump is run at its maximum rate and the oxygen is introduced as rapidly as possible without loss from the open end of the silica tube. The sample (of foodstuff, for instance) is placed on a piece of sheet iron, 5 cm. wide and 76 cm. long, which is introduced into the heated silica tube at such a rate that it burns completely in the oxygen (without soot or tar formation). If soot is formed the analysis is not ruined but the apparatus must be washed out and the washings evaporated and burned again. In order to prevent an explosion the sample is lighted with a gas pilot flame at the moment of introduction. In order to prevent the sample from burning outside the tube it is placed in separate piles on the sheet iron; and if all cannot be placed on one piece, the rest is placed on other pieces of sheet iron which are introduced in succession until the whole sample is burned. The heat applied to the outside of the silica tube must be very high in order to complete the burning of all the carbon, and is conveniently supplied from a row of large Méker burners. An asbestos roof over the tube is used to decrease the loss of heat. The Cottrell precipitator is useful in precipitating fine particles of ash that would not be caught by the wash bottles.

Fig. 2.

At the end of the ashing, the ash is removed to a ball mill and water is used in rinsing the sheet iron "boats," the Cottrell precipitator and silica tube, and this, together with the sodium hydroxide solution in the absorption flask and wash bottles, is evaporated to a small volume (2–5 cc.) and analyzed for iodine.

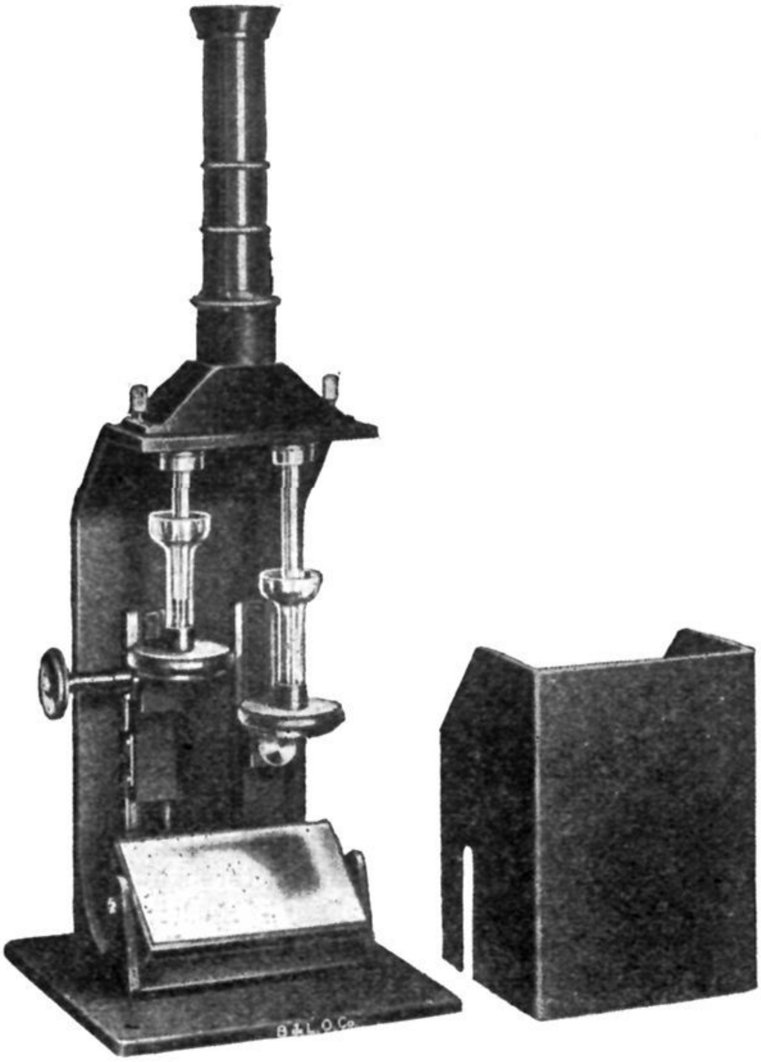

Fig. 3.

The ash is extracted with water or if it contains a large amount of water soluble salts with absolute alcohol (free from aldehyde) in a ball mill. Sufficient alcohol is used to permit decantation after the extraction, and the extraction is repeated several times. The combined extracts are filtered and evaporated. The residue is dissolved in about

2 cc. of water and analyzed separately or after addition to the sodium hydroxide solution and washings.

In the iodine analysis, the solution is neutralized with phosphoric acid (to which $^1/_5$ volume of 0.1 N sulfurous acid has been added) using an indicator paper made by drying an alcoholic solution of methyl orange or brom-phenol blue on ash-free filter paper. It is heated to boiling to expel carbon dioxide and sulfur dioxide and cooled. The volume is now made up to 10 cc. and the solution transferred to a 12cc. separatory funnel (preferably with a 10 cc. graduation mark for making up to volume). One cc. of purified carbon tetrachloride and 1 or 2 mg. of sodium nitrite are added and the separatory funnel is shaken 100 times; during the shaking $^{85}/_{95}$ of the iodine should pass into the carbon tetrachloride and color it pink or violet, so that by multiplying the yield by 1.118 the total iodine in the sample may be calculated. The carbon tetrachloride is cloudy with water droplets and is run into a 1cc. glass-stoppered bottle and centrifuged in the special centrifuge head shown in Fig. 2. It is then run into the left cup of the Bausch and Lomb micro-colorimeter shown in Fig. 3 and set at 20 mm. Carbon tetrachloride containing pure iodine of the concentration 0.1 mg. per cc. is placed in the right cup and a color match made. Ten readings are made and their average is taken. The reading in millimeters divided by 200 and multiplied by 1.118 gives the milligrams of iodine.

It is well for each person using the method to check his technic in the extraction of the iodine by taking 10 cc. of distilled water containing 0.1 mg. of iodine in the form of iodide in the separatory funnel, adding phosphoric acid to the turning point of the indicator paper, a small crystal of sodium nitrite and 1 cc. of carbon tetrachloride, and shaking 100 times. In analyses in which there is more than 2 mg. of iodine it will precipitate in the water and delay extraction and if the amount of iodine is very large its solubility in carbon tetrachloride may be a factor limiting extraction so that a series of repeated extractions is necessary. In this case the iodine in each extraction is determined separately in the colorimeter and the amount in the last extraction only multiplied by 1.118. Then the various amounts are added together to obtain the total.

In the analysis of drinking water much time may be saved by adding sodium carbonate and filtering out the alkaline earths during the evaporation. Only 2% of the iodine is lost by filtering a 100-liter sample boiled down to 1 liter (after adding 1 g. of sodium carbonate). Some organic matter is carried down in the precipitate and probably holds tenaciously some of the iodine. Although the iodine content of the sludge of sewage purification plants is much higher than that in the run-off (on the basis of the same water content), showing that the iodine is concentrated in the organic precipitate (in the bodies of live bacteria or dead material), in the case of drinking water the amount of organic matter is very much less, in fact comparatively insignificant, and the loss due to filtering off some of the organic matter is negligible. The organic matter remaining in drinking water after boiling and filtering is usually so low that a smaller tube (of Pyrex glass) may be used in the combustion and the precipitator dispensed with.

In the case of soils, combustion in the tube is desirable before proceeding with the analysis, and the hydrochloric acid-soluble portion may be analyzed according to the procedure outlined above. The biological significance of the analysis of the hydrochloric acid-insoluble portion (fused with sodium hydroxide) is not apparent as it is doubtful if it is available to organisms.

An average of 10 readings with the Duboscq colorimeter is about as accurate as one reading on a buret and there is no occasion for verifying the colorimetric determination by means of subsequent titration, as done by Von Fellenberg.[6]

Technical carbon tetrachloride may be used in the analysis if oxidized with chlorine or bromine in sunlight, washed with sodium hydroxide solution and water, dried with plaster of Paris, and distilled, rejecting the cloudy portion of the distillate.

If any ash that comes in contact with the hot silica tube is removed with hydrofluoric acid before the tube is used again, its life will be prolonged. It might be economical to use a smaller tube. The rate of combustion is limited by the size of the pump and precipitator. The original tubes were 4 ft. long and were found to be too large.

Since 0.001 mg. is about the limit of iodine that may be determined in the micro-colorimeter, it is necessary to have more than 0.01 mg. for accurate work. The iodine content of many samples of foodstuffs is so low that it may be necessary to burn several kilos in one analysis. The absolute accuracy in such extreme cases is somewhat doubtful, but the general value of the method is attested by the following results. Starch was purified until no iodine could be detected in it by the method, then to a 50-gram sample was added a known quantity of iodine in the form of a potassium iodide solution. The results were as follows.

IODINE IN MILLIGRAMS

	1	2	3
Added	0.045	0.20	0.090
By analysis	.046	.19	.093

In order to show the relative value of the different features of the combustion method, a much larger sample of iodide was added to starch and the ash and various fractions of volatilized iodide were analyzed separately with the following results.

	Total iodine added to starch, %
Ash	44.4
Large absorption flask	47.0
Precipitator	5.7
1st wash bottle	1.4
2nd wash bottle	0.7
Total recovered	99.2

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[6] Von Fellenberg, *Biochem. Z.*, **152,** 116 (1924).

In the above experiment the silica tube was heated only sufficiently to burn the sample, but when it is heated to a high temperature, the iodide is sometimes completely volatilized, none remaining in the ash. In case the ash is bulky and alkaline, it retains more of the iodine. In burning thyroids some of the iodine appeared to sublime as I_2 and it is suggested that in case organic matter does not reduce all of the iodine to iodide, a little sulfur be added to the sample.

Although the errors by this method may be 1% on samples containing more than 1 mg. of iodine and 5% on samples containing less than 1 mg. of iodine, it was shown by McClendon and Rask[7] that the method of Kendall, which is accurate for 0.5 g. of thyroid or 100 g. of blood, entirely fails in the analysis of iodine in Minnesota wheat, some samples of which contain only 1 mg. of iodine per metric ton of wheat (McClendon and Hathaway).[8] Attempts were made to compare the method with that of Von Fellenberg but I did not acquire sufficient skill with his method to be confident of the results. Since his colorimetric determinations are made under the microscope, special skill is required.

Some refinements of technique were worked out in collaboration with Gertrude Humphrey Beckwith[9] in relation to her work on the iodine content of water supplies. Appreciation is due to Don R. Mathieson for assistance in some of the experiments and to the American Medical Association for a grant for purchasing some of the apparatus.

Summary

Traces of iodine are determined colorimetrically in a micro-colorimeter made on the Duboscq principle. If organic matter is present in the sample it is burned in oxygen in a tube and the combustion products are sucked through sodium hydroxide solution and a Cottrell precipitator. The limit of error is 1% on samples containing more than 1 mg. of iodine and 5% on samples containing less than 1 mg. A sample of foodstuff as large as several kilograms can be used in one determination; hence the process is possible where most other methods entirely fail.

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[7] McClendon and Rask, *Proc. Soc. Exptl. Biol. Med.*, **20**, 101 (1922).

[8] McClendon and Hathaway, *J. Am. Med. Assocn.*, **82**, 1668 (1924).

[9] Beckwith, *Proc. Soc. Exptl. Biol. Med.*, **25**, 117 (1927).