

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE SOUTH CAROLINA FOOD RESEARCH COMMISSION, AND THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF MINNESOTA MEDICAL SCHOOL]

THE DETERMINATION OF TRACES OF IODINE. V. FURTHER REFINEMENTS IN TECHNIQUE

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In previous papers of this series¹ there has been developed a technique for the micro-estimation of iodine in organic materials. Although the methods as described have been employed in a number of laboratories, further improvement seemed possible at certain points in the operation. It has been found practically impossible to avoid the formation of some tar or soot if organic matter is burned in the silica tube by the method described in the second and third papers, where the sources of heat were the heat of combustion of the material itself and a bank of Méker burners placed under the tube. The production of tar and soot necessitates a second burning, in a similar or smaller tube. Low temperature ignition in a muffle as previously described possesses the advantage of brevity, so essential in survey work where a large number of samples must be analyzed, and gives fairly accurate results on plant materials (except grain). Reith reports losses as high as 60% by this method when applied to flour or milk, but our results on milk powder have been quite as good as by any other method. It cannot be applied to nuts, cheese or other oily substances, which must be burned in the tube. Careful temperature control by automatic pyrometers is extremely necessary. In the fourth paper a small oxygen-gas torch was described, which insures sufficient heat to incinerate the sample thoroughly, and at the same time an excess of oxygen. Since this torch worked well on the small samples used in the determination of iodine in thyroid tissue, it seemed desirable to attempt to adapt it to reinforcing the heat of combustion of the larger samples necessary when foods or excreta are burned. Heat outside the silica tube as by Méker burners cannot be relied upon to maintain the stream of gases above the ignition point of organic combustion products, even though an excess of oxygen is always present. This external heat has therefore been replaced by a "hot spot" inside the tube over which the gases must pass, the hot spot consisting either of an electrically heated platinum spiral placed in a narrow part of the tube, or a roughly coiled piece of chromel wire placed a short distance in advance of the oxygen-gas torch. The platinum spiral has been found particularly adapted to the burning of such substances as urine and feces, which must be placed in combustion boats, while the flame-heated chromel wire has been found quite as satisfactory, and much simpler

¹ McCleendon and co-workers, *THIS JOURNAL*, 50, 1093 (1928); 51, 394 (1929); 52, 541 (1930); 52, 980 (1930).

in operation, for those substances which can be fed continuously, such as powdered dried vegetables by a screw feed device, or oils by an atomizer.

The flame-heated spiral inside the tube becomes very hot.² We have frequently been troubled with the appearance of nitrite in our absorbers, even when substances low or lacking in nitrogen, such as starch, were burned. This we have attributed to the fixation of atmospheric nitrogen, and have avoided by partially closing the opening around the screw feed device at the end of the tube, and so adjusting suction and oxygen supply that very little air is drawn in. Any appreciable amount of nitrite ruins the determination, since iodine is set free when the solution is acidified. The use of sodium sulfite instead of or in addition to hydroxide in the absorbers has been found desirable. Even so, it is a question whether all iodate is reduced by sulfite in alkaline solution, so that in some cases we have found it desirable to adopt the suggestion of Reith³ to acidify and add sulfurous acid, and then make alkaline again before evaporation to complete dryness. Iodates are not extracted by alcohol. Reith also holds that alcohol will not make a complete extraction of iodides from a semi-crystalline mass, due to inclusion in the crystals. By making extraction in a ball mill, however, (which he did not do) and so adjusting our concentration of alcohol that a two-phase liquid system is formed, we are able to obtain satisfactory recoveries.

For burning any substances which can be continuously fed, such as oils by an atomizer or powdered substances by a screw-feed device, the set-up is essentially as previously described⁴ except that the Méker burners are omitted, and the platinum spirals replaced by about 75 cm. of 20 gage chromel wire roughly coiled into a conical spiral and placed inside the silica tube about 5 cm. in advance of the point of delivery of the sample. To heat the spiral, two small copper tubes (1.5 mm. bore) are attached to the outside of the feed device, to deliver oxygen and gas. The ends of these tubes are brought together to form a torch immediately in front of the delivery point of the sample. The joint between the feed device and the silica tube is covered with a wad of wet asbestos, to exclude air as far as possible. The supply of oxygen is so adjusted as to furnish practically all the gas that is drawn through the absorbers. Burning is complete and extremely rapid, requiring only twenty minutes for 100 g. of material. A curved piece of 22 gage sheet nickel large enough to catch the ash, if inserted inside the silica tube, will facilitate removal of ash and lengthen the life of the tube. After the combustion, adhering particles of ash are

² Platinum wire was tried for the spiral, but fused completely (m. p. 1755°). Tungsten wire (m. p. 3400°) was tried but ignited. The chromel wire becomes oxidized, but holds its form. Its life, however, is only one burning.

³ J. F. Reith, *Biochem. Z.*, 224, 223 (1930).

⁴ McClendon, Remington, von Kolnitz and Rufe, *THIS JOURNAL*, 52, 542, 544 (1930).

removed from the wire, which is preferably not placed in the solution, the tube and piece of nickel washed, washings combined with the solutions from the absorbers and the determination carried out as directed in previous papers. In burning oils, it is not practicable to feed both torch and atomizer from the same cylinder of oxygen. To check recovery when fats are burned by this method, we analyzed a sample of cod-liver oil, finding 10,400 parts of iodine per billion, and a sample of butter fat, finding 40 parts. The two were then mixed in proportions varying from 1 to 25 to 1 to 10, and burned. Percentage recoveries were, respectively, 80, 90, 80, 100, 91, 87, 84, 83, 82; average 86.

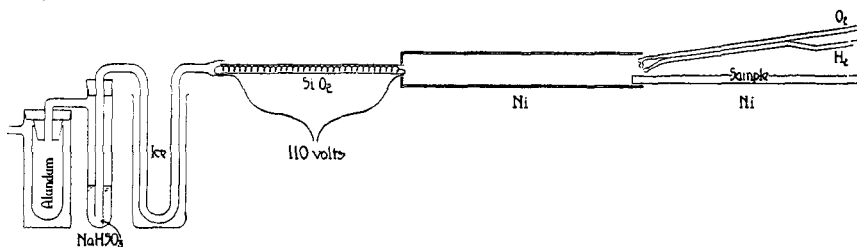


Fig. 1.

To burn urine residues and other pasty materials which cannot be packed into sausage casings nor sprayed, a different set-up has been devised (Fig. 1). This consists of a nickel tube of convenient diameter, into which a long nickel boat containing the sample is introduced. Nickel is used to reduce breakage when substances containing considerable amounts of fusible alkali, such as urine, are burned. The end of the nickel tube is constricted so as to fit over a silica tube of about 5 mm. bore, which contains a platinum spiral. This spiral is heated to a cherry red electrically by means of a 110-volt alternating current through an adjustable transformer. The joint between the nickel and silica tubes need not be perfectly tight but should be fitted fairly snugly. It is not necessary that the platinum wire be insulated from the nickel, but the whole apparatus can be placed on a dry wood or alberene table and the operator protected from accidental contact by wrapping the torch with tape. One end of the silica tube is introduced into the end of the U-shaped Pyrex tube, the joint being packed with wet asbestos. This tube is immersed in a beaker of cracked ice, and is connected with one or more Milligan wash bottles filled with sodium bisulfite solution. Finally the gases are drawn through an alundum capsule or Jena fritted glass filter to catch the white smoke which sometimes appears and is not absorbed by the wash solution. The nickel boat containing the sample is introduced gradually, the sample being burned by the small oxygen-gas torch shown in the drawing, which has a small opening in the oxygen supply tube, so placed as to allow a part of the oxygen to escape into the combustion tube above the sample. After the

sample has all been introduced and burned, the torch is removed, the opening at the end is partly closed, oxygen being introduced continually, and the whole of the nickel tube is heated by means of a burner, so as to burn or volatilize any matter which may have been deposited in it during the earlier stage of the combustion. The washings from the apparatus and the liquid from the absorption vessels are combined and treated as previously described.

Pfeiffer⁵ has developed an acid distillation method involving oxidation by perhydrol in sulfuric acid. We have tried the modification of this method proposed by Glimm and Isenbruch⁶ but the operation is extremely time-consuming, is not adapted to large samples (in the tube furnace we have burned as high as 1 kg. of wheat or corn continuously), the oxidation is never complete, and the results are low in our hands. Out of more than twenty trials we were able to recover measurable quantities of iodine in only three. A sample of dried whey, which was the only one to give results comparable to the tube method, was carbonized in a dish before the acid digestion. A few typical results by these different methods are given in Table I.

TABLE I
SOME COMPARATIVE IODINE ESTIMATIONS BY DIFFERENT METHODS
Results are in parts per billion, dry basis

Sample	Source	Low tempera- ture muffle	Acid distillation	Tube combustion	
Skim milk	Orangeburg, S. C.	371	354	316	
Skim milk	Newberry, S. C.	657		646	
Skim milk	Greenville, S. C.	840		862	
Skim milk	Edgefield, S. C.	169	166	314	
Skim milk	Greenwood, S. C.	1000		1300	
Whey	Greenwood, S. C.	1090	1070	1612 ^a	1768 1646
Cottonseed oil	Kershaw, S. C.			59	
Cottonseed meal	Kershaw, S. C.	111	104		
Cottonseed hulls	Kershaw, S. C.	111	117		
Oysters	McClellanville, S. C.		4,560	15,400 16,200	
Osborne-Mendel salt mixture contg. citrates and a small amt. of iodide soln.			10,600	26,000 26,000 24,000	

^a Charred preliminary to acid digestion.

Turner⁷ has recently described a method for estimating iodine in blood, in which he employs ashing in a current of oxygen in an open crucible, oxidation with bromine, the use of the iodide-iodate reaction, and colorimetric measurement of the blue color developed with starch. Although we have not worked on blood, we have repeatedly found that losses occur if ashing is done in open dishes at uncontrolled temperatures, even when an

⁵ G. Pfeiffer, *Biochem. Z.*, **215**, 126 (1929).

⁶ E. Glimm and J. Isenbruch, *ibid.*, **207**, 368 (1929).

⁷ R. G. Turner, *J. Biol. Chem.*, **88**, 497 (1930); *THIS JOURNAL*, **52**, 2768 (1930).

excess of alkali is used. Neither can we confirm his observations as to the reliability and stability of the color developed. If the blue solution is read against a blue glass instead of a starch-iodine solution, it will be found that the color is quite sensitive to temperature changes, and varies with time. Furthermore, in our hands, the depth of color produced by a given amount of iodine was not uniform. Four solutions, each prepared as described by Turner for his standard solutions, and each containing 0.001 mg. of I_2 , read 22.5, 12.8, 9.24 and 26.0 mm. in the colorimeter, against our blue glass. Apparently there is some uncontrolled factor.

Other workers have made use of the iodide-iodate reaction to increase the iodine available for estimation. We have always felt that due to the uncertainty as to the presence of oxidizing substances other than iodate in extracts of the ash of plant and animal matter, and the effect of atmospheric oxygen on free hydriodic acid, this is a very dangerous procedure. It would be very desirable to increase the sensitivity of our present methods for substances such as blood or tissue, of which small amounts only are available, and for cereals or diets extremely low in iodine. Atmospheric effects can perhaps be eliminated by promptly removing the liberated iodine from the excess of potassium iodide by shaking out with carbon tetrachloride. This would move the lower limit of estimation down to about one-sixth its present value. When this was tried with pure solutions prepared by oxidation of known quantities of sodium iodide, the results were quite constant, and tended to approach the theoretical value, usually somewhat less. When, however, it was applied to extracts prepared from the ash of plant or animal matter, results were found to be extremely variable, and often many times the amount we had found to be present by other methods. Alcohol cannot be supposed to be an absolutely selective solvent for iodides to the exclusion of all other oxidizable substances.

Summary

1. An improvement in the destruction of organic matter in the micro-estimation of iodine by the tube furnace method, consists in a "hot spot" within the tube over which gaseous products must pass. This makes a second combustion unnecessary.
2. The perhydrol-sulfuric acid distillation method of Glimm and Isenbruch was found to be inapplicable to large samples of plant or animal matter, giving incomplete oxidation and low results.
3. The starch-iodide reaction is unreliable as a quantitative colorimetric method for estimation of iodine.

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