

# Oxygen Flask Method for Iodine Determination in Thyroid Protein

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**Nitrogenous oxidation products formed during the combustion of thyroid powder give rise to an appreciable error in the oxygen flask method for total iodine as described in the British Pharmacopoeia. This error can be reduced to negligible proportions by the use of a sulfamic acid reagent and an increased period of stirring of the combustion residue before the addition of excess iodide and titration with thiosulfate.**

**T**HE OXYGEN flask combustion method as applied to halogen determination in organic material (1) was modified by Vickers and Johnson (2) for the analysis of iodine in certain pharmaceuticals. More recently this method has been adopted by the British Pharmacopoeia (3) for iodine determination in thyroid. Vickers and Johnson mentioned that the presence of nitrogenous oxidation products in their method was undesirable. This communication describes the magnitude of the error thus caused in thyroid iodine measurements and assesses the effectiveness of sulfamic acid as used by Backer (4) to eliminate it.

## EXPERIMENTAL

**Equipment.**—A Thomas-Lisk combustion flask, black wrappers with fuse, magnetic stirring apparatus with Teflon covered bar and ring as supplied by A. H. Thomas Co., Philadelphia, Pa., or similar equipment.

**Reagents.**—Prepare reagent solutions as described by Vickers and Johnson (2), using water which has been distilled over alkaline permanganate.

**Bromine Solution.**—Dissolve 100 Gm. of potassium acetate in glacial acetic acid, add 4 ml. of bromine, and dilute to 1 L. with glacial acetic acid.

**Sulfamic Acid.**—Dissolve 4 Gm. of sulfamic acid in 200 ml. of water.

**Sodium Thiosulfate.**—Standardize accurately to about 0.005 *N*.

**Formic Acid.**—88%  $H_2CO_2$ .

**Starch Indicator.**—0.5% in water.

**Procedure.**—Accurately weigh an amount of thyroid containing 0.2–0.5 mg. of iodine and enclose in a black paper wrapper. Use not more than 100 mg. of protein material when combustion is to be performed in a 1-L. flask. With larger amounts, combustion was often incomplete. Add 10 ml. of water and 2 ml. of *N* NaOH to the flask.<sup>1</sup> Place the magnetic stirring bar in the bottom of the flask and thoroughly flush the flask with 100% oxygen. After combustion of the thyroid powder, stir the residue for 15 min. rather than 5 min. as required in the B. P. procedure. Add 5 ml. of bromine reagent and let stand for 2 min. Add 0.5 ml. of formic acid and flush out the bromine vapors with air. Carefully wash down the sides of the flask with 15 to 20 ml. of water, add 2 ml. of sulfamic acid reagent, and allow to stand a further 2 min. Finally, add 1 Gm. of

crystalline potassium iodide to the flask and titrate immediately with 0.005 *N* sodium thiosulfate using starch indicator as the end point is approached.

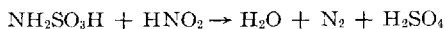
For combustion of standard amounts of *o*-monoiodobenzoic acid (MIB) accurately prepare alcoholic solutions containing about 10 mg. of MIB/ml. Apply carefully measured aliquots of these solutions directly on the black wrappers. Prepare dry mixes in mortars containing nicotinic acid and urea, respectively, and 80–100 mg. of MIB/10 Gm. total weight. For the preparation of combustion packets weigh the dry mixes or thyroid powders directly on the wrappers, enclose, and place in the platinum basket with wick protruding.

## RESULTS AND DISCUSSION

**Carbon Dioxide and Iodine Determination.**—There was no appearance of free iodine or evidence of erroneous recoveries of added iodate when, following the usual procedure, 100 mg. of lactose was burned in a 120-mg. black paper wrapper. Carbon dioxide, and other oxidative residues which may result from the wrapper, did not therefore seem to produce an error.

**Nitrogenous Oxidation Products of Urea Combustion.**—Urea, if it is chemically pure, will yield only water, carbon dioxide, and nitrogenous oxides during combustion. Table I illustrates the extent of oxidation of iodide by nitrogenous products of urea combustion in this method. When an amount of urea containing 32 mg. of nitrogen ( $\equiv 200$  mg. of protein) was burned and the combustion residue stirred for 5 min. there appeared 0.20 meq. of free iodine following addition of excess iodide. When the stirring time was increased to 15 min. the amount of iodine appearing at the time of titration was reduced to 0.002 meq. Similarly, if sulfamate were added to the combustion residue after a 5-min. stirring period and the usual bromine treatment, the amount of free iodine appearing at the time of titration was also reduced to about 0.002 meq. When the longer stirring period was combined with the sulfamate treatment in the prescribed manner, there was no detectable iodine present at the time of titration.

It is reasonable to presume that at least some of the gaseous nitrogenous products of combustion will form nitrites in solution. Sulfamic acid reacts with nitrite in the manner indicated by the equation (5):



In view of the effectiveness of the sulfamic acid in prevention of the undesirable iodide oxidation, it seemed probable that the part of the nitrogenous oxidation products, which was present as, or could give rise to nitrites, caused most or all of the error. As confirmation of this hypothesis, a sensitive test

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<sup>1</sup> With the use of a magnetic stirring apparatus and a 1-L. round-bottom flask it has been found that the alkalinity and volume constituted by 10 ml. of water and 2 ml. of *N* NaOH is adequate to capture the iodine products of combustion. The B. P. procedure employs a 50-ml. volume of water in a 2-L. flask and 2 ml. of *N* NaOH. Excluding this minor departure, the term "B. P. method" in this text is as described in the "British Pharmacopoeia," 1963, p. 835.

TABLE I.—EFFICIENCY OF SULFAMIC ACID IN REMOVAL OF NITROGENOUS OXIDATION PRODUCTS WHICH OXIDIZE IODIDE TO FREE IODINE

Urea Nitrogen, mg.	Time Between Addition of KI and Titration, min.	Iodine, meq.			
		5 min. Stirring After No Sulfamate	Combustion Sulfamate	15 min. Stirring After No Sulfamate	Combustion Sulfamate
32	2	0.20	<0.002	<0.002	No detectable iodine
32	5	0.21	0.002	0.002	
32	10	0.21	0.002	0.002	
32	30	0.21	0.002	0.003	
32	60	0.22	0.002	0.004	

TABLE II.—THE RECOVERY OF IODINE FOLLOWING COMBUSTION OF *o*-MONOIODOBENZOIC ACID (MIB., 51.17% IODINE)

MIB Combustion Prepn.	Mix Wt., mg.	Wt. MIB, mg.	Theoretical Iodine, mg.	Recovered Iodine, mg.	% Recovery
Alcoholic soln., 1.01 mg. MIB/ml.	...	0.101	0.0517	0.0497	96.0
...	...	0.202	0.1034	0.1025	99.2
...	...	0.404	0.2067	0.1870	90.4
...	...	0.606	0.3102	0.2740	88.2
...	...	0.808	0.4136	0.3760	91.0
...	...	1.010	0.5170	0.4370	84.5
...	...	5.050	2.5840	2.1450	83.0
Urea	53.7	0.430	0.2220	0.2210	99.5
MIB	71.4	0.572	0.2960	0.2835	95.8
Dry mix, 0.8055 mg. MIB/100 mg.	45.2	0.368	0.1905	0.1850	97.2
Nicotinic acid	101.2	1.068	0.552	0.551	99.8
MIB mix, 1.067 mg. MIB/100 mg.	97.2	1.037	0.531	0.507	95.6
...	99.1	1.058	0.541	0.527	97.6

for the presence of nitrite was performed on combustion residues. By this method, if nitrite is present in the test solution the diazonium derivative of sulfanilic acid is formed and couples with  $\alpha$ -naphthylamine giving a red-colored solution (6). In this manner, nitrite was demonstrated to be present in urea and thyroid powder combustion residues both before and after treatment with bromine, notwithstanding a greatly diminished color intensity in the latter instance. After treatment of the combustion residues with sulfamic acid in the prescribed way, no nitrite could be detected by this method.

The recommended procedure, therefore, included the addition of sulfamate as well as the longer stirring period for the combustion residue. The longer stirring period evidently allowed the flask solution to reach a new chemical equilibrium which favored the disappearance of nitrite.

**The Recovery of Iodine from Combustion of MIB.**—When alcoholic solutions of MIB were applied to the black paper wrappers and the combustion and iodine measurement carried out in the recommended manner, it was possible to recover only about 90% of the theoretical amount (Table II). The recoveries of iodine under these conditions were observed when the iodine present on the wrapper ranged from 0.1 to 2.5 mg. The fluctuating recovery values suggested that combustion of the wrapper and MIB did not uniformly convert all the iodine to an inorganic form. When the combustion of MIB was carried out in the presence of organic nitrogenous material, as represented by urea and nicotinic acid, there was a distinct increase in re-

coverable iodine. Recoveries under these conditions ranged from about 96% to the theoretical value. Evidently, the slower burning, which would be expected with the greater bulk of organic material, allowed the iodine to reach an inorganic state before burning ceased.

**Iodine Determination in Thyroid Powders.**—Four different thyroid samples were analyzed for their iodine content by the (a) U.S.P., (b) B.P. (3) or Vickers-Johnson, and (c) B.P. modified procedures. The results are shown in Table III. The B.P. modified procedure included the 15-min. stirring period and the addition of sulfamic acid. The U.S.P. procedure (7) employs a carbonate ashing step at 675 to 700°, bromine treatment to convert all iodine to iodate, subsequent acidification with phosphoric acid, and expulsion of excess bromine by boiling. Traces of remaining bromine are organically bound by addition of a phenol solution, and the iodine is measured by a thiosulfate titration. With the proposed modifications in the oxygen flask procedure, the observed iodine levels are indistinguishable from those given by the U. S. P. method. These results indicate that, under the prescribed conditions, oxygen flask combustion of thyroid combined with bromine treatment of the residue, converts the organically bound iodine to iodate, and retains it in that form at least as efficiently as the U.S.P. method. Iodate is the required chemical state of iodine before addition of excess iodine and thiosulfate titration.

The B.P. method gave as much as 15% higher values than those obtained by the authors' modified

TABLE III.—A COMPARISON OF RESULTS OF IODINE ANALYSIS ON THYROID POWDERS AS DETERMINED BY THE U.S.P. AND OXYGEN FLASK PROCEDURES<sup>a</sup>

Thyroid Sample	U. S. P. XVII Procedure	Oxygen Flask	
		B. P.	B. P. Modified
1	0.970	1.14	1.01
	0.977	1.16	1.02
	0.960	1.17	1.01
		1.08	1.00
2	0.730	0.864	0.740
	0.748	0.849	0.714
		0.812	0.718
		0.796	0.728
3	0.196	0.220	0.206
	0.206	0.226	0.207
	0.203	0.234	0.206
		0.240	0.209
4	0.516	0.632	0.549
	0.543	0.678	0.545
	0.560	0.644	0.543
		0.583	0.555

<sup>a</sup> All concentrations are expressed as per cent by weight iodine.

method. The difference, it is contended, is due to the spurious oxidation of iodide by nitrite. Furthermore, the noticeably greater variation between individual determinations may possibly be due to the recurring end point which is a characteristic of the B.P. method.

**Recovery of Iodide from Thyroid Combustion Residues.**—Iodine was added as potassium iodide to a thyroid powder combustion residue immediately after the 15-min. stirring period, and the iodine determination was carried out in the usual manner.

TABLE IV.—THE RECOVERY OF IODINE ADDED TO A THYROID POWDER COMBUSTION RESIDUE

Thyroid (0.548% I), <sup>a</sup> mg.	Iodine, mg.—			% Recovery
	From Thyroid	Added as KI	Recovered	
50.8	0.278	0.200	0.476	99.5
48.7	0.266	0.200	0.466	100.0

<sup>a</sup> This iodine concentration is the average of the 4 determinations listed for sample 4 in Table III.

Table IV demonstrates that the recovery of the added iodine was quantitative, and, therefore, it appears that no combustion product of thyroid affected the titration conditions.

## SUMMARY AND CONCLUSIONS

The British Pharmacopocia method for the determination of iodine in thyroid was modified by the adoption of a 15-min. stirring period for the combustion residue and later addition of sulfamic acid to remove nitrites. With these modifications, the iodine levels obtained in thyroid powder analyses were comparable to those obtained by the U.S.P. procedure.

These measurements were performed on amounts of thyroid containing between  $1.5 \times 10^{-6}$  and  $6 \times 10^{-6}$  Gm. equivalent iodine. Tomlinson (8) has drawn attention to the error in halogen determination at this concentration due to high blank values caused by the wrapping material. It was demonstrated that the wrappers employed here do not give a blank value. He also pointed out that combustion may be incomplete by the very nature of the combustion operation. This was not in evidence in this investigation where sample size was restricted to 100 mg. or less and where the iodine constituted between 0.2 and 1.0% of the total organic substance burned. However, when amounts of MIB containing comparable quantities of iodine were burned with the wrappers only (120 mg.), the iodine could not be quantitatively recovered. The combination in thyroid powders of organic material and iodine in what appears to be fortuitous proportions, makes the oxygen flask method for iodine determination in thyroid most attractive. This procedure can be completed in 30 min. and there is no transfer of solutions.

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