deposits obtained. Separation from lead is satisfactory if the control potential is not above −0.30 volt *vs.* S.C.E.

The method is distinctly more rapid than the conventional one, total time for reduction and deposition of weights of copper from about 175 to about 800 mg. being from 20 to 30 minutes. This is to be compared with about 45 minutes for the conventional method plus 15 or 20 minutes additional time for reduction with hydrazine or hydroxylamine hydrochlorides.

The writer's students have performed a number of satisfactory analyses on brass samples as large as 1 gram, which are not reported here. A minor disadvantage is the dilution of the solution with the anolyte. This amounts to 50 ml., at most, and the author has not found it serious.

One rough determination of current efficiency was made by noting the current at intervals, plotting this against time, and integrating graphically. A value of 96% was obtained.

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# Distillation of Micro Quantities of Iodine

## *Application to Determination of Protein-Bound Iodine in Bovine Blood Serum*

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THE catalytic effect of iodine on the rate of reaction between cerium(IV) and arsenic(II) first described by Sandell and Kolthoff (*8*) and adapted to a colorimetric procedure by Chaney (*3*) has proved very satisfactory for the determination of the small quantities of iodine found in blood serum. After oxidation of the organic matter and of the iodine to iodate with a mixture of sulfuric and chromic acids, the iodine is distilled after the addition of a suitable reducing agent. In this distillation much time can be saved if the volume of distillate can be kept low, and Chaney (*3*) has described a widely adopted procedure by which this may be accomplished. The apparatus, however, is fairly complex, and gives somewhat erratic recoveries approximating 85%.

The borosilicate glass distillation apparatus illustrated in Figure 1 is simple in design, and with it more consistent recoveries approximating 96% are obtained (see Table I). Both aeration and distillation are utilized to effect rapid transfer of the iodine to the distillate, thus keeping the volume of distillate small.

The application of this still to the determination of the protein-bound iodine in bovine serum is described below. The protein precipitant of Somogyi (*9*) is used to separate the protein-bound iodine which consists largely if not completely of thyroxine iodine (*12*). This method is widely used (*1, 7, 10, 11*).

The widely used chromate oxidation procedure (*3, 6*) is modified by using a stream of air to aid in defining the point at which heating should be stopped. In the colorimetric determination, chloride ions are used to increase the sensitivity (*1, 2, 4, 5, 8*).

### REAGENTS

**Iodine-Free Water.** Add 1 gram of sodium hydroxide to 3 liters of distilled water and redistill in all-glass still.

**Arsenious Acid.** Dissolve 2.636 grams of C.P. arsenious trioxide in a solution of 150 ml. of water and 13.4 ml. of sulfuric acid (sp. gr., 1.84) by heating on a hot plate, cool, and make to a volume of 200 ml.

**Ceric Ammonium Sulfate.** Dissolve 12.65 grams of the C.P. salt in a hot solution of 125 ml. of water and 19.6 ml. sulfuric acid (sp. gr., 1.84), cool, and make to a volume of 200 ml.

**Chromic Acid.** Dissolve 300 grams of chromium trioxide in 300 ml. of water. (Grasselli's chromium trioxide was found to be low in iodine.)

**Phosphorus Acid.** Boil Baker's C.P. 50% phosphorus acid for 30 minutes, maintaining constant volume by adding water from time to time.

**Sodium Arsenite.** For a 0.1 *N* solution, dissolve 0.2166 gram of sodium arsenite in 50 ml. of solution.

**Sodium Chloride.** Make solution containing 20 grams per liter.

**Sodium Hydroxide.** Use a 0.75 *N* solution and a 1% solution.

**Concentrated Sulfuric Acid.** Add 10 ml. of concentrated hydrochloric acid per liter of C.P. sulfuric acid and boil for 30 minutes.

**70% Sulfuric Acid (by Weight).** Slowly add 470 ml. of sulfuric acid (sp. gr., 1.84) to 270 ml. of water.

**Zinc Sulfate.** Dissolve 12.5 grams of zinc sulfate heptahydrate in 125 ml. of 0.25 *N* sulfuric acid and make up to a volume of 1 liter.

**Iodine Standard.** Dissolve 0.0327 gram of C.P. potassium iodide (dried overnight at 135° C.) in water, and make up to 250 ml.; dilute 1 to 1000 for working standard of 0.1γ iodine per milliliter.

### PROCEDURE

**Precipitation of Protein-Bound Iodine.** Add 32 ml. of the zinc sulfate solution to 4 ml. of serum in a 50-ml. centrifuge tube and

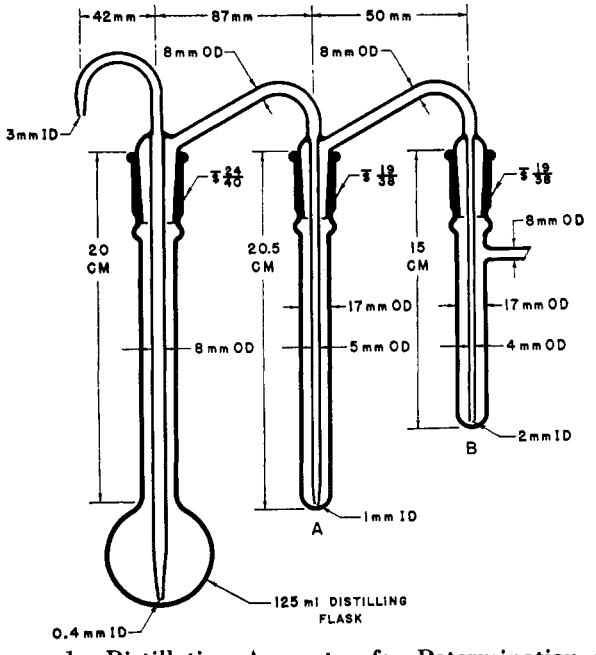


**Figure 1. Distillation Apparatus for Determination of Iodine**

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**Table I. Recovery of Iodine from Bovine Blood Serum**

| Sample | Aliquot of Distillate | % T, 34° C., 20 Min. | Iodine in Aliquot, γ | Iodine, Total, γ | Recovery, % |
|---|---|---|---|---|---|
| 4 ml. serum + 1 ml. $H_2O$ | 3 | 84.0 | 0.094 | 0.470 | .. |
| | | 84.5 | 0.095 | 0.475 | .. |
| | 3 | 83.5 | 0.094 | 0.470 | .. |
| | | 83.0 | 0.093 | 0.465 | .. |
| 4 ml. serum + 0.4 γ I[a] | 1 | 55.0 | 0.057 | 0.855 | 96.3 |
| | | 54.0 | 0.056 | 0.840 | 92.5 |
| | 1 | 55.5 | 0.058 | 0.870 | 100.0 |
| | | 55.0 | 0.057 | 0.855 | 96.3 |
| 4 ml. serum + 0.8 γ I | I | 75.5 | 0.083 | 1.25 | 97.5 |
| | | 74.5 | 0.082 | 1.23 | 95.0 |
| | 1 | 74.5 | 0.082 | 1.23 | 95.0 |
| | | 74.5 | 0.082 | 1.23 | 95.0 |
| [b] | 1 | 76.5 | 0.084 | 1.26 | 98.8 |
| | | 76.5 | 0.084 | 1.26 | 98.8 |
| [b] | 1 | 75.0 | 0.082 | 1.23 | 95.0 |
| | | 74.5 | 0.082 | 1.23 | 95.0 |
| | Water blank | 10.0 | | | |
| | 0.05 γ I | 52.5 | | | |
| | 0.10 γ I | 87.0 | | | |

[a] Added as KI.
[b] Distillation time, 15 minutes.

stir. Add 4 ml. of 0.75 *N* sodium hydroxide solution in 1-ml. portions while swirling the tube by hand. Stir vigorously with an electric stirrer after all 4 ml. have been added. Centrifuge 10 minutes at 2000 r.p.m. Decant the supernatant liquid, add 25 ml. of iodine-free water, and resuspend the solid matter using the stirrer, and again centrifuge. Wash a total of four times in this way, and dissolve the residue in 5 ml. of 70% sulfuric acid. Heat may be required to accomplish complete solution.

**Digestion of Sample.** Transfer the solution quantitatively to the distillation flask using a total of 25 ml. of concentrated sulfuric acid to rinse out the centrifuge tube. Add 4 ml. of chromic acid solution and a large carborundum chip and heat the flask on a sand bath to a temperature of 200° to 220° C.

In this laboratory samples are run in sets of eight, and the flasks are heated on individual sand baths, 10 cm. in diameter and 2 to 2.5 cm. deep, containing a layer of sand about 0.5 cm. deep. Heat is supplied by Fisher burners operated at maximum heat intensity. The heating serves a two-fold purpose of completing the oxidation of organic material and of the iodine. Decomposition of excess chromic acid occurs as the temperature rises above 180° C.

The end of the heating period is determined by passing a stream of air into the flask for about 10 seconds, starting about 5 minutes after the digestion mixture has begun to froth. Repeat the procedure at 1- to 2-minute intervals until faint white fumes persist after the removal of the air current. Further heating should be avoided because chromous salts may precipitate, causing serious bumping and possible loss of sample.

After cooling below 100° C., add 15 ml. of water and boil using the same method to determine the point at which the heating should be stopped.

**Distillation of Iodine.** Add 1 ml. of 1% sodium hydroxide and 3 drops of sodium arsenite to absorption tube *A* and 0.5 ml. of the sodium hydroxide to tube *B*. Dilute the contents of the digestion flask with 15 ml. of water and place it on a sand bath heated by a low flame. Draw a stream of air through the still by attaching a vacuum line to the side arm of tube *B*. The rate of air flow is very important and is controlled by a flowmeter.

A simple but effective flowmeter can be made by affixing T-seals on both ends of a U-tube having upright limbs 25 cm. high and 13 cm. apart and inserting a capillary tube 4.5 cm. long with a 2-mm. bore between the limbs. The capillary tube is held in place with two sections of rubber tubing which permits the use of capillaries of different bore diameters for establishing the rate of flow. Flowmeters employing the same principle can be obtained from Fisher Scientific Co. The rate of flow should be about 1500 ml. per minute. The flowmeters should be calibrated against a wet test meter or some other type of flowmeter known to be accurate. If the rate of flow exceeds 1500 ml. per minute by very much, the iodine values will be too high, and the distillate will be colored green. The green color is probably due to the mechanical transfer of chromium salts from the flask to the absorption tube (*14*). Carr *et al.* (*2*) found that chromic ions catalyze the reaction between cerium(IV) and arsenic(III), and it is likely that this ion is responsible for the high results. Too low a rate of flow will result in low iodine values due to incomplete distillation of the iodine.

Heat the distillation flask, and when the contents reach a temperature of 80° to 90° C., draw two 1-ml. portions of 50% phosphorus acid into the flask by placing the acid in a 3-ml. microbeaker and sucking the acid in through the bubble tube of the flask. Next, draw three 1-ml. portions of arsenious acid into the flask and continue the distillation for 10 minutes starting from the second addition of phosphorus acid. Combine the distillates from tubes *A* and *B* and make up to a final volume of 15 ml.

**Colorimetric Determination of Iodine.** Add 0.5 ml. of arsenious acid and 0.5 ml. of sodium chloride solution to a 3-ml. aliquot of the distillate in a colorimeter tube 18 mm. in diameter which has been cleaned with hot concentrated nitric acid or a solution of Nacconol (Allied Chemical & Dye Corp., N. Y.) and sodium pyrophosphate. Place the tubes in a water bath maintained at 34° ± 0.5° C. After 15 minutes in the bath, add 0.5 ml. of ceric ammonium sulfate solution. The acidity of the solution should be between 0.4 *N* and 1.5 *N* at this point. Read the samples in an Evelyn type colorimeter after a reaction time of 20 minutes, using Corning filter 5543 (554) (standard thickness). Prepare standard curves for each set of determinations at levels of 0.00, 0.05, and 0.10 microgram of iodine.

## DISCUSSION

A large part, if not all, of the iodine is distilled as free iodine, and Barker (*1*), Talbot *et al.* (*10*), and Thomas *et al.* (*13*) recommended using a reducing agent in the absorption chamber to effect more complete absorption. Barker uses sodium sulfite and Talbot *et al.* sodium bisulfite, both of which must be removed before the catalytic reaction is carried out. Sodium arsenite as recommended by Thomas *et al.* was found to be equally effective and has the advantage that it need not be removed.

During the oxidation with chromic and sulfuric acids, unidentified volatile substances are formed that interfere with the colorimetric reaction and in the usual procedure are removed by boiling off two portions of water. When a stream of air is used, as described, to aid in controlling the course of this oxidation, enough of these interfering substances are removed so that a single portion of water is sufficient. Acetate ion does not interfere with this method as it does with that of Trevorrow and Fashena (*14*).

## SUMMARY

A new type of still and certain modifications of Chaney's procedure for the determination of iodine in blood serum are described. Consistent results with recoveries of 96% are obtained.

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