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# A One-Day Modification of the Dry Ash PBI Method

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Melting point determination with a carbon arc image furnace is unique in that there is no container problem. Since the energy of the furnace is concentrated in a small area the specimen forms its own container eliminating a common source of error.

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# A One-Day Modification of the Dry Ash PBI Method

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*with the technical assistance of Donna Douglas*

*Abstract.* A modification of the dry ash method for the protein bound iodine content of plasma or serum has been devised. This method permits completion of the determination in one laboratory day and produces results that compare favorably with the original two-day method. Recovery experiments were satisfactory and indicate no loss of iodine in the drying, ashing, or colorimetric procedures in the method.

A method commonly employed for the analysis of the protein bound iodine content of serum or plasma was devised by Barker, et al, in 1950. The procedure was developed in this university and in modified form has been used in our clinical protein bound iodine laboratory since its inception. The method essentially consists of the precipitation of the plasma or serum proteins, washing of the precipitate, drying in the presence of sodium carbonate, ashing, dissolving the ash in an acid solution and colorimetrically measuring the disappearance of the color of ceric ammonium sulfate in the presence of sodium arsenite. Although many modifications of the method have been proposed, the time consuming overnight drying of the precipitate in the presence of sodium

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carbonate results in a method that requires two days for its completion.

The present paper is concerned with a study of the attempts to shorten this drying period to permit complete analysis in a working day.

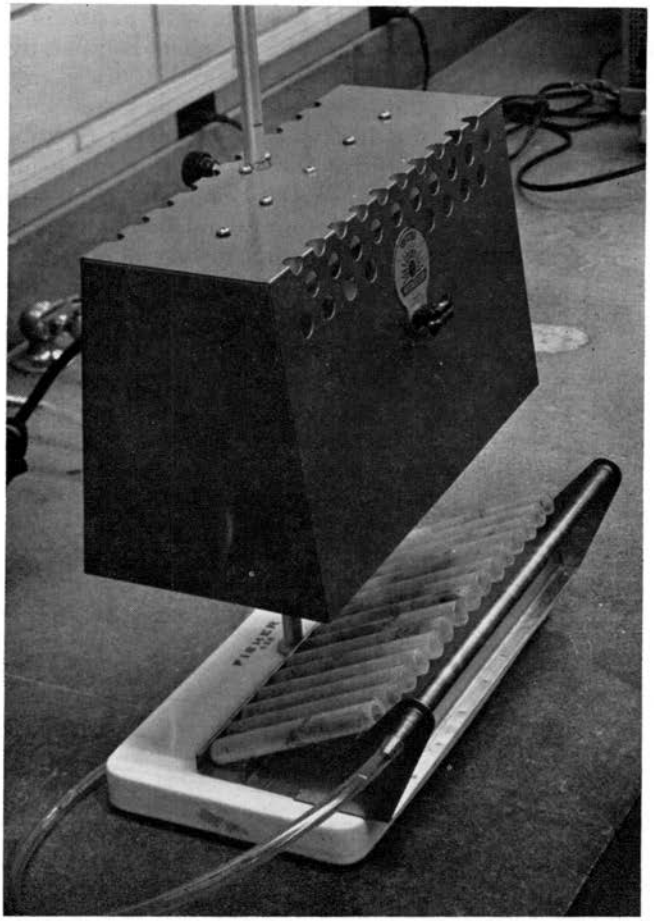

Figure 1. Intfra-red lamp drying device.

*Experimental*:

Over ten years ago experiments were devised to reduce the time required by the PBI method. The tubes containing the precipitated plasma proteins plus the sodium carbonate solution were placed in a heated sand bath in a vertical position. The drying time could be reduced to about three hours, but even with constant attention the contents of some of the tubes would boil out the top. Heating blocks at 100° C. also dried the tubes in

about 3 hours with occasional boiling over. Since a 3 hour period of drying plus the required 2½ hours in the muffle furnace left insufficient time for the precipitation, washing and colorimeter procedures, attempts were made to further shorten the drying period. In the Clinical Chemistry Laboratories of the Mayo Clinic infra-red lamps are mounted over the tubes which are placed in a rack in an inclined position with a stream of air directed down the center of the tube. A similar apparatus was constructed in our laboratory (Figure 1) and its use resulted in a more rapid drying of the contents of the tubes. Several shortcomings were observed in this type of equipment. It was very difficult to adjust the heights of the lamps to produce uniform heating without the contents of some tubes boiling over. Also, the tubes in the rack did not dry uniformly and it required rotation of the tube positions to achieve rapid drying of all tubes. To overcome the difficulties of this procedure we again used cast aluminum heating blocks at 100° C. and found that it required about 3 hours to dry 20 tubes without attention. A special rack was constructed that fit over the top of the drying block and directed a fine stream of air into the center of each vertically held tube (Figure 2). The temperature of the block was increased to 106° C. and employing aslow stream of air the contents of 20 tubes could be dried within 60 to 90 minutes. The air flow through holes drilled with a No. 50 drill was found most effective at 80 ft. per minute.

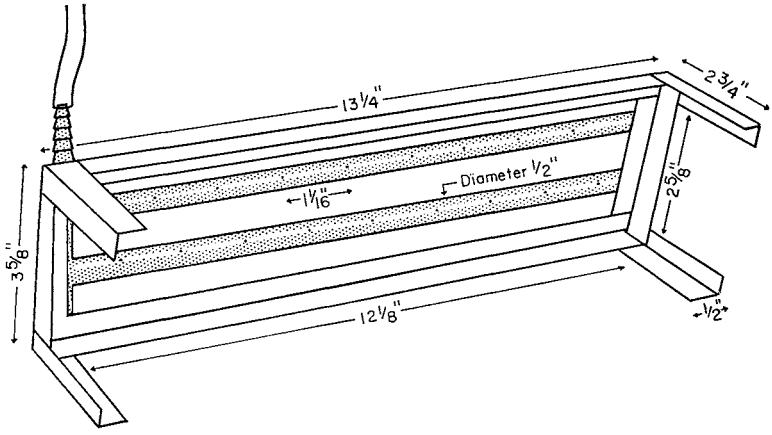


Figure 2. Aeration rack.

*Method:*

The one-day method in use in our laboratory involves several modifications of the original procedure (1,2) and will be presented in detail.

*Precipitation*:

Specimens of serum or plasma, 1.0 ml., in duplicate, are diluted with 7 ml. of distilled water using a 10 ml. serological pipette with a large orifice. The 1 ml. portions of zinc sulfate and sodium hydroxide solutions are then added to precipitate the proteins. The tubes are covered with a piece of parafilm and the contents are thoroughly mixed by inversion.

*Washing*:

After centrifugation, the supernatent fluid is discarded and the precipitate is washed once with 10 ml of water. Thin footed stirring rods are used to completely suspend the precipitate. Other methods recommend multiple washings to remove inorganic iodides that may be present in the serum or plasma. In an extensive study of the washing process in our laboratory we found that a single washing removed inorganic iodides and that further washings failed to remove contamination from organic iodides that precipitated with the proteins. At the completion of the washing process the tubes are centrifuged and the supernatent fluid is discarded.

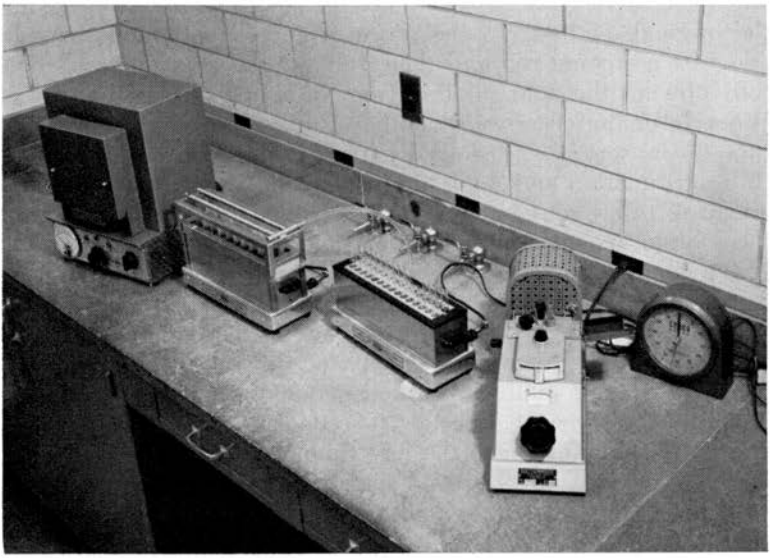

Figure 3. Instruments used for PBI determination. From left to right are the Thermolyne muffle furnace, the dri-bath with aeration rack in place, the special dri-bath containing 30 holes fro Klett colorimeter tubes, the Klett colorimeter tubes, the Klett colorimeter and timer.

The muffle furnace, 106°C. Dri-Bath and 30 hole 39°C. Dri-Bath were kindly furnished by Thermolyne Corporation, Dubuque, Iowa. The aeration rack, furnace and Dri-Baths as well as a muffle furnace holding 80 tubes and a small air compressor are available from Thermolyne.

*Drying*:

The 1 ml. of sodium carbonate solution is added to the washed

 

precipitate, the contents are mixed thoroughly with a Vortex stirrer, and the tubes are dried for 60 to 90 minutes in the dry bath set at 106° C. with a stream of air directed into the tubes as shown in Figure 3. The shortest drying time may be achieved by bringing the contents of the tubes to a boil before starting the air flow and turning off the air 10 to 15 minutes before the tubes are completely dry. Some experience is required to establish a procedure for drying the tubes in approximately 60 minutes.

*Ashing*:

The dry tubes are transferred to a muffle furnace which has been preheated to 600° C., and are ashed for 2½ hours at that temperature. The preheating of the muffle furnace saves time and is conveniently carried out by turning on the furnace when the tubes are started in the drying process.

*Colorimetry*:

The tubes are removed from the muffle furnace, cooled, and the HCl-$H_2SO_4$ solution is added to dissolve the ash. This solution is a mixture of HCl and $H_2SO_4$ (23 ml. conc. HCl and 56 ml. conc. $H_2SO_4$ per liter) that contains in a 7 ml. volume the same amount of acid that was contained in the two 2 ml. aliquots in the original method. To the series of tubes containing the ash add first a stirring rod and then 3 ml. of the acid mixture. The rods prevent the acid solution from bubbling out of the tubes. When bubbling ceases add another 4 ml. of the mixture to each tube in the series. Undissolved particles of carbon are separated by centrifugation and 3 ml. aliquots of the supernatent fluid are added to tubes containing 1 ml. of distilled $H_2O$ and 0.5 ml. of the sodium arsenite solution. A maximum of 24 tubes per batch (Klett colorimeter tubes) are placed in a special dry bath, Figure 3, containing 30 holes and maintained at 39° C. The extra six holes are used to preheat tubes containing the ceric ammonium sulfate solution. When the contents of the tubes have reached 39° C. (5 to 10 minutes), ceric ammonium sulfate solution is added to the series of tubes at 30 second intervals. Twelve minutes after the addition to the first tube the colors are read in a Klett colorimeter at 30 second intervals. If more than 24 tubes have been ashed, this process may be repeated until all the colors are read.

*Results*:

Since our laboratory currently carries out approximately 100 PBI determinations a week, we have had ample opportunity to compare the results of the one-day method with those obtained by our earlier modification of the procedure. Essentially, the "old" two-day method referred to in Table I consisted of precipitation and washing procedures carried out on the first day with over-

Table I
Comparison of typical results obtained by the one-day versus the two-day PBI method.

| Control Serum 1* | | Control Serum 2* | | Patients' Serum* | | |
|---|---|---|---|---|---|---|
| 2 day | 1 day | 2 day | 1 day | Specimen | 2 day | 1 day |
| μg/100 ml | μg/100 ml | μg/100 ml | μg/100 ml | | μg/100 ml | μg/100 ml |
| 4.7 | 5.0 | 10.7 | 10.6 | A | 3.6 | 4.0 |
| 5.0 | 5.2 | 9.3 | 9.7 | B | 6.4 | 6.1 |
| 4.7 | 5.0 | 10.5 | 10.6 | C | 4.0 | 4.2 |
| 5.1 | 5.0 | 10.2 | 9.7 | D | 5.0 | 5.4 |
| 4.7 | 5.2 | 10.6 | 10.6 | E | 4.7 | 4.7 |
| 4.4 | 4.7 | 9.7 | 9.7 | F | 1.3 | 1.3 |
| 4.4 | 4.4 | 10.3 | 10.2 | G | 10.2 | 10.2 |
| 4.7 | 4.7 | 10.1 | 10.2 | H | 2.3 | 2.6 |

*Range 4.5-5.1 μg/100 ml. *Range 9.4-10.6 μg/100 ml *Patients from Thyroid Clinic

night drying of the precipitates in a forced draft oven. Ashing and the colorimetric procedures were carried out on the second day with a water bath at 39°C. employed in colorimetry. Typical results taken from our files are shown in Table I to illustrate the similar results obtained by both methods. Recovery experiments in the original method of Barker, et al, (2) consisted of the addition of known amounts of iodine in the form of sodium iodide thyroxin or thyroglobulin to tubes containing precipitated and washed plasma proteins. To check the present method through the drying, ashing, and colorimetric phases, a known amount of iodine in the form of sodium iodide was added to the sodium carbonate solution prior to the drying process. Table II presents typical results of these recovery experiments.

Table II
Recovery experiments obtained from the one-day PBI method.

| Specimen | Serum Value μg/100 ml | Iodine Added μg/100 ml | Total Calc'd. μg/100 ml | Total Found μg/100 ml | Recovery % |
|---|---|---|---|---|---|
| A | 3.2 | 4.1 | 7.3 | 7.7 | 105.6 |
| B | 2.9 | 4.1 | 7.0 | 7.2 | 102.9 |
| C | 8.9 | 4.1 | 13.0 | 13.2 | 101.6 |
| Patients D | 7.0 | 4.1 | 11.1 | 11.4 | 102.7 |
| from E | 4.7 | 4.1 | 8.8 | 9.1 | 103.5 |
| Thyroid F | 6.2 | 4.1 | 10.3 | 10.6 | 102.9 |
| Clinic G | 18.7 | 4.1 | 22.8 | 22.1 | 97.0 |
| H | 5.4 | 4.1 | 9.5 | 9.4 | 99.0 |
| I | 5.0 | 4.1 | 9.1 | 9.0 | 98.9 |
| J | 6.5 | 4.1 | 10.6 | 10.5 | 99.0 |
| Control | 4.7 | 4.1 | 8.8 | 8.9 | 101.2 |
| Serum | 5.0 | 4.1 | 9.1 | 9.3 | 102.2 |
| Range 4.7-5.1 | 4.7 | 4.1 | 8.8 | 9.1 | 103.5 |
| Control | 10.6 | 4.1 | 14.7 | 14.8 | 100.7 |
| Serum | 10.6 | 4.1 | 14.7 | 15.3 | 104.0 |
| Range 9.4-10.6 | 11.4 | 4.1 | 15.5 | 15.8 | 102.0 |

*Discussion:*

The protein bound iodine content of several thousand plasma and serum specimens has been determined in our laboratory

since the publication of the dry ash method (1,2). For several years the results of each batch have been controlled by the simultaneous determination of standard control sera. This continuous experience has enable an adequate comparison of modifications resulting in the one-day method. The typical results presented in Table I point out the similar values obtained by both methods using control sera with different PBI levels and patients sera. The recovery experiments in Table II were designed to check every procedure in the method that would retain added inorganic iodide. It can be seen from these experiments that iodine was not lost in the more rapid drying process at 106° C.

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# Sulfonation of Bathophenanthroline and Bathocuproine

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4,7-Diphenyl-1, 10-phenanthroline, introduced in 1952 as a spectrophotometric reagent for iron by Smith, McCurdy and Diehl (1), has proved useful and popular; some twenty papers (2) have now appeared detailing its application to the determination of iron in sea water, wine, serum, urine, various metals, and other materials. This reagent was given the common name Bathophenanthroline. The great sensitivity of the reagent is made peculiarly useful by virtue of the solubility of its red, ferrous derivative in isoamyl alcohol, for extraction of the red compound from water into the immiscible solvent provides a concentration method making possible the determination of iron in concentrations as low as 0.002 parts per million, and even more important, provides a method of removing the iron contaminants from the various reagents used in the analysis.

The one disadvantage bathophenanthroline suffers is the occasional appearance of a turbidity when the analysis is performed entirely in the acqueous solution, especially when perchlorate is present. The English chemist Trinder (3) solved this problem by sulfonating bathophenanthroline by treatment with chlorosulfonic acid, the sulfonated product being completely water soluble, even in the presence of perchlorate. Later Blair and Diehl (4) isolated and characterized the sulfonated

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