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Mercury Analysis in Biological Material by Direct Combustion in Oxygen and Photometric Determination of the Mercury Vapour

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Mercury in small quantities in, for example, biologic material is determined by direct combustion in oxygen of samples weighing between 20 and 200 mg. The combustion gases are passed through different zones under temperature conditions carefully controlled to allow complete oxidation of organic material, but also to avoid the formation of mercury oxide. The mercury is temporarily collected on a gold filter. When all combustion products have passed through the system the mercury absorbed on the gold is released by heating, and the mercury vapour is determined photometrically by means of ultraviolet light absorption at 253.7 nm.

The reproducibility of the method is good; the difference between duplicate analyses is mostly less than 10 %. Samples containing about 1 ng of mercury per g may be analysed without difficulties.

In the analysis of mercury in samples of biologic origin, photometric determination of mercury vapours has frequently been utilized, for example, by Lindström,[1] Jacobs *et al.*,[2] Nielsen Kudsk,[3] and Ulfvarson.[4] Up till now the methods of isolating the mercury from the samples have been rather complicated and uncertain.

In the method of Jacobs *et al.*,[2] the samples were digested with sulphuric acid and potassium permanganate and the digested samples extracted with dithizone in chloroform. The chloroform solution was then evaporated in an ignition tube and, finally, the mercury was liberated by heating with a flame, and then determined photometrically when passing a gas cell.

In the modification developed by Ulfvarson,[4] the digestion of the sample and the evaporation of the mercury is according to Jacobs *et al.*,[2] but then the mercury is absorbed on gold and again rapidly released by heating the gold. This technique makes it possible to concentrate the mercury vapour and to avoid influence by other compounds which absorb light at the same wavelength as mercury.

The present method utilizes the principle of concentrating the mercury vapours and eliminating the background influences by absorbing the mercury on gold, but combined with direct combustion of the sample in oxygen. This simple and straightforward technique is made possible by using small samples and passing the products of the first combustion through different zones with carefully controlled temperature conditions.

EXPERIMENTAL

Apparatus

The apparatus is shown in Fig. 1. It comprises a combustion tube of quartz, internal diameter 13 mm. In the middle of the tube there is a constriction 40 mm long, with an internal diameter of 5 mm. The straight part, 500 mm long, contains a 120 mm filling of quartz wool close to the constriction. The other part, about 400 mm long, is bent V-shape and contains sodium carbonate (small grains), with a plug of quartz wool at the blow-out end. The combustion tube is closed with a glass stopper at the blow out end. The V-shaped part is immersed in a bath of metal (60 % tin and 40 % lead) kept at a constant temperature of 294—298°C. The sodium carbonate column has to be filled in such a way that the highest part can be lowered not less than 20 mm below the level of the melted metal (not important at the blow-out end). The side arm at the beginning of the combustion tube is connected *via* gold filter I to a container for compressed oxygen. Gold filter I has to prevent introduction into the apparatus of small amounts of mercury always present in the gas. Filter I is made of a quartz tube 190 mm long, internal diameter 4 mm, and with a constriction in the middle. A 23—25 mm long filling of about 0.5 mm gold grains is placed between two plugs of quartz wool.

A cooling tube is attached to the blow-out side arm by means of heat-resistant teflon tubing (ordinary rubber tubing is used for other connections). The cooling tube is of pyrex glass, 500 mm long, with an internal diameter of 7 mm except at the connection ends, and bent U-shape. It is held nearly horizontal, with the bent part about a centimeter higher than the connection ends. Next to the cooling tube there are two cold traps, cylindrical vessels of pyrex glass about 105 mm long and 30 mm in diameter. The inlet tube has to end at about half the distance from the bottom of the vessel.

Cold trap I is held in a horizontal position and is only air cooled; trap II hangs vertically with half its length immersed in water at 10°C during combustion of samples.

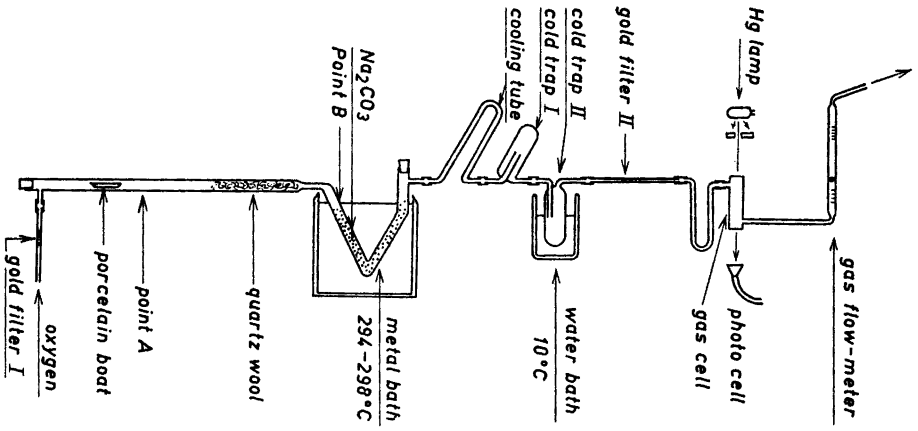

Fig. 1. Sketch of the apparatus for the microdetermination of mercury.

Gold filter II is attached to the blow-out tube of trap II. It is the same size as gold filter I. The quartz wool plugs are, however, replaced by a plug of slightly sintered gold grains; and the filter is mounted in a sloping position to keep the gold column compact. (The sintered gold plug is made by heating to redness in the tube gold grains kept together by a cotton plug. Cotton rests are rinsed out with a stream of air and heating).

Gold filter II is connected with the gas cell in a photometer, by a glass tube not less than 500 mm long, diameter 3 mm, with its first part bent U-shape.

Finally, a flowmeter, measuring range 100—2000 ml/min, is connected to the gas cell.

Photometer. The instrument is a Beckman DU spectrophotometer fitted with a 100 mm long gas cell, diameter approximately 15 mm. The light source is a mercury lamp, wavelength adjusted to 253.7 nm. Signals from the photomultiplicator are recorded by means of a Beckman recorder, fitted with a Beckman scale expander. The sensitivity of the instruments should be sufficient to obtain about 4 mm high absorption peaks for 0.2 ng mercury introduced according to the procedure described below. (Corresponding to about 0.3 % absorbance according to the experiments made.) The sensitivity range is controlled by a filter of optical quartz, 4 mm thick, which was found to have a light absorption at 253.7 nm corresponding to that of about 12—15 ng of mercury.

Accessories. Heat reflector: a 90 × 70 mm steel plate bent semicircular and used as a movable roof about 20 mm over the combustion tube. Electric heating tape, 25 mm wide. Porcelain boats, volume 1—1.5 ml.

Remarks. Special care must be taken to free the apparatus from mercury. The fillings should be heated in an electric furnace for several hours: quartz wool at 800°C, sodium carbonate at 700°C and the porcelain boats for a shorter time at 1000°C.

Procedure

A suitable amount of the sample is weighed into a porcelain boat. The heating tape, adjusted to 85 ± 5°C, is wound four turns, side by side, around gold filter II. Cold trap II is immersed in water to half its length. The quartz wool section of the combustion tube is fitted with a cooling jacket of wet cotton wool. The boat is placed in the combustion tube about 120 mm in front of the quartz wool. The gas container valve is opened and a stream of oxygen passed through the apparatus at the rate of 50 ml/min. Heating is started (with a large Meker burner) about 10 mm behind the boat at point A, Fig. 1. (In the following all the movements are indicated by the direction of the gas stream.) The flame is moved slowly backwards (to the boat), but when the sample catches fire the burner is moved forwards and kept at point A until the burning ceases. Slowly heating in this way will permit the sample to burn with a minimum of smoke and sublimation. The remainders in the boat are heated to redness with the reflector placed over the tube. At this stage all the escaped organic products are trapped in the quartz wool together with condensed water. The water has to be removed first. This is achieved by removing the cooling jacket and stroking with the flame several times backwards the quartz wool section of the tube. The organic remainders are burnt and removed in two stages. First, the burner is held under the quartz wool section close to the metal bath until the lowest part of the tube becomes red; then the flame is moved 40 mm backwards, held there as before, and so on, until the whole contaminated part of the tube has been heated. At the last stage the burner is placed at point A with the reflector above, and the heating arrangement is moved forwards, slowly enough to heat the tube and its contents to redness. Finally, the constriction and the bent part of the tube close to the level of the metal in the bath, are also heated (point B, Fig. 1). The oxygen flow rate is increased to 1500 ml/min. Water, which has condensed in the cooling tube (this applies to samples weighing more than about 50 mg), evaporates and is partly condensed in cold trap II. Rinsing is continued until the cooling tube, cold trap I, and the inlet tube inside trap II are completely dry, or for not less than 3 minutes. The recorder should now draw a straight zero line (otherwise one has to wait for about a minute). The gas rate is adjusted to 250 ml/min (see discussion), and the heating tape is removed from gold filter II. The filter is heated to dull redness, and here it is important to let the flame surround the whole gold section at once. The height of the recorder response is measured and the corresponding amount of mercury is calculated by means of a calibration graph. Gold filter II is cooled with water and disconnected from cold trap II. Any water left in cold

trap II is expelled by a stream of oxygen and by heating with a flame. The procedure takes about 30 minutes, depending to some extent on size and character of the sample.

Once daily the mercury, gathered on gold filter I, is expelled by heat and rinsed out with oxygen.

Calibration

Solutions. Mercury standard solution: 135.4 mg of mercury (II) chloride are dissolved in 0.25 N sulphuric acid and diluted to 100 ml with the same acid. The solution contains 1.00 mg Hg per ml.

Dithizone solution: 12 mg of dithizone, free from mercury, are dissolved in 1 liter of chloroform. If stored in a refrigerator (darkness) the solution can be used up to one month.

Preparation of standards. 1.00 ml of the mercury solution is diluted to 100 ml with double-distilled water.

250 ml of 0.25 N sulphuric acid are transferred to a separatory funnel (volume about 400 ml; the stopcock not lubricated and without stem). Any contaminating mercury is removed from the acid by shaking twice with dithizone solution, followed by two shakings with chloroform, using 10 ml portions each time.

1.00 ml of the diluted mercury solution is now added to the acid. The mercury is extracted twice with 10 ml portions of the dithizone solution by vigorously shaking, and once with 10 ml of chloroform. The extracts are taken up in a 200 ml volumetric flask and diluted to the mark with chloroform. One ml of the solution contains 50 ng of mercury. Different volumes, corresponding to 1.5, 2.5, 5, 10, 15, 20, 25 ng of mercury, are transferred to porcelain boats by means of an "Agla" micrometer buret and allowed to evaporate to dryness. The samples are stable for about one month when stored cold.

Another calibration series should be prepared if the samples contain more than 500 ng of mercury per gram. Four ml of the standard solution are taken instead of one, diluted and extracted as described, giving a final chloroform solution with 200 ng of Hg per ml. From this solution a series of samples is prepared containing 10—200 ng of Hg.

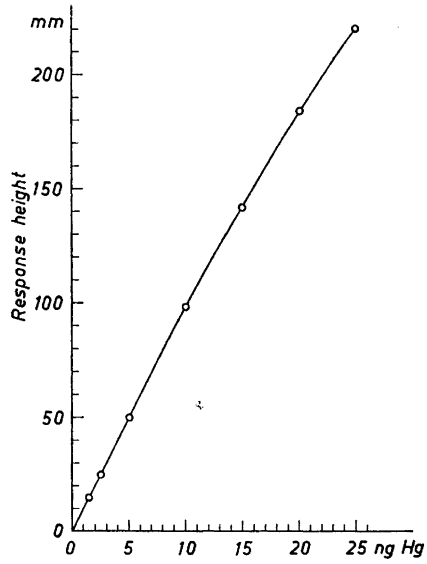

Fig. 2. Standard curve.

To make sure that no additional mercury is received from the reagents, 20 ml of the dithizone solution are diluted to 200 ml with chloroform. 0.5 ml of this solution are evaporated in a boat and run as a blank.

Standard analysis. Analysis of the standard samples is simpler than the main procedure described above. The quartz wool section of the combustion tube and cold trap II are not water cooled. The boat is heated with the reflector on, from the beginning (at a gas flow rate of 170 ml/min). The heating arrangement is then moved directly forwards (at a gas flow rate of 250 ml/min), thus excluding the two other steps in the main procedure. After rinsing 3 min at a gas flow rate of 1400 ml/min, the analysis is completed by heating gold filter II at a gas flow rate of 250 ml/min.

DISCUSSION

The dimensions of the apparatus and the rather complicated procedure have been developed empirically to obtain optimum conditions for the combustion and separation of mercury. Organic substances cannot be burnt up completely by a single heating in an oxygen stream. Some components and intermediate products, often containing mercury, are distilled off and, if not destroyed, will seriously contaminate the apparatus. Other arrangements, such as passing the combustion gases through columns of oxidizing agents at higher temperatures or burning samples in a pressure chamber, have not been found suitable since mercury oxidizes at temperatures above 300°C which, among other difficulties, obstructs a quantitative absorption on gold. In the procedure described the temperature may be quite high when the sample is burning, but, the organic constituents may act as reducing agents at the slow oxygen flow rate to counteract any oxidation of mercury, and, moreover, liberated mercury will immediately be removed from the high temperature zone. The residues from the first combustion, which sublimate in the quartz wool, are oxidized by the subsequent heating procedure. An excess of oxygen is essential for the combustion and, therefore, water is separated from the organic remainders in the quartz wool by careful preheating. If intense heat is applied here at once, water will temporarily supplant the oxygen, thus causing the combustion to be incomplete.

Finally, any residues, which escape both combustion steps, are assumed to be transformed into harmless products in the hot sodium carbonate column. This column works very satisfactorily when not too large amounts of organic sublimates are introduced, the bath temperature is kept between the given limits, and the gas flow rate is not increased. At bath temperatures, a few degrees above 300°C, some mercury will be lost in the determination. On the other hand, too low bath temperatures and/or a too high gas flow rate will cause incomplete final oxidation of the organic material.

The cooling tube, together with both cold traps and the heating tape on gold filter II constitute an arrangement for the separation of mercury from water and gaseous combustion products.

Experiments have shown that gold filter II can be warmed up to 90°C without influencing mercury absorption. On the other hand, when kept at room temperature the good filter adsorbs water together with other gaseous products, which would contaminate the gold surface, causing poor mercury adsorption and a false response from the photometer in the following step.

Table 1. Control analysis. The values are given in ng Hg per gram, each value representing the result from a single determination.

Sample	Hg by activation technique	Amount of sample taken, mg	Hg by combustion-photometric procedure
Blood	3.2	377	3.7
		482	3.7
Blood	3.5	326	3.1
		429	3.3
Blood	7.3	308	8.5
		394	8.6
Blood	13	312	12.2
		424	12.9
		233	12.4
Blood	20	212	22.7
		290	22.0
		214	22.5
		230	22.5
Blood	96	225	100
		150	99
		200	97
Plasma	2.2	383	1.8
		516	1.8
Plasma	6.6	344	5.8
		404	6.2
Plasma	6.7	246	6.5
		414	5.9
Plasma	81	151	79
		222	75
Plasma	110	103	122
		135	132
Plasma	265	60.8	265
		76.9	260
Urine	2.5	192	2.1
		208	1.7
Egg-yolk	15	136	12.5
		186	13.4
Egg-yolk	250	71.7	255
		84.8	256
Egg-yolk	250	92.0	273
		60.2	275
		50.1	270
White of egg	32	117	28
Liver	87	206	54
		71	55
Liver	460	25.2	484
		41.4	530

The cooling of cold trap II with water is essential to establish a low enough water vapour pressure in the gas when passing the warm gold filter II. Cooling also enables the final mercury determination to be made before all the water is expelled from the apparatus. Shortly after the inlet tube becomes dry the remaining water collects as a droplet in the lowest part of the cooled vessel, and thus a low, constant vapour content is obtained, as indicated by a steady

background absorption in the photometer, somewhat above the zero level. Experiments with water added to standard samples, and with repeated analysis of blood with all the water expelled, and with water left in cold trap II, showed that the low, constant vapour absorption does not influence the height of the mercury peaks.

The optimal gas flow rate for the final determination is dependent on the size and properties of the gold filter and the subsequent parts of the assembly. The optimal gas flow rate for a new apparatus has to be established by analysing known samples (standard samples with the same mercury content). Mercury is determined at different gas flow rates. The gas flow rate giving the maximum peak height is chosen as the most suitable.

The heating program previously described was found to be the most suitable for blood and urine. For other organic samples other heating programs, established by experiments may be preferred.

Control analysis. In order to investigate the practical value of the method a number of analyses were made, using various samples of biologic origin. These samples have also been analysed for mercury by a neutron activation technique at Isotoptekniska laboratoriet, Stockholm, Sweden. The results are shown in Table 1.

As can be seen from the results there is no systematic difference between the two methods. The reproducibility seems to depend somewhat on the possibility of obtaining homogeneous and representative samples. The use of too large samples should, however be avoided since they are more time-consuming and require special care in connection with combustion. Generally, samples of 50 to 200 mg are recommended. If very low concentrations of mercury have to be analysed, higher photometer sensitivity and scale expansion are preferable to larger samples.

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