Determination of Microquantities of Iodine in Biological Materials

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This paper presents a modified method of iodine determination in biological material using the results of various authors and own knowledge. For our procedure the iodine mass loss was experimentally determined using radionuclide ^{125}I. The average value of iodine mass losses 19.8 % indicates that the method is suitable for the determination of microquantities of iodine (10—400 μg dm^{-3}) in biological materials.

Iodine was discovered by Courtois in 1811 and belongs to the most important biogenic elements. Its presence in the organism is essential for the normal function of thyroid gland and so the attention of medicine has been focused to this fact.

Various continental plants and animal organisms including human beings got in contact only with very small amounts of iodine. Geochemical data show that iodine content in the earth crust, including sea and atmosphere accounts for 6×10^{-6} % of the total mass of the Earth. The richest source of iodine is considered Chilean alum which contains 0.2 % of iodine in the form of sodium iodide. For a long time the negligible amounts of iodine in biological materials hindered the exact research of its natural cycle and metabolism in the organism. In addition, the insufficient accuracy of the analytical methods used contributed to such situation.

The intake of iodine into the human organism by regular food is very low, especially in the countries located far from the sea. Under such conditions a majority of iodine originates from the iodized salt which, however, does not always fulfill the requirements for optimal intake of iodine.

Within the last decade the growing attention is focused on the examination of iodine balance in man. The easiest way is the estimation of its content in the urine. Though it is indisputable that the iodization of table salt improved the severe iodine deficiency in this country, the present day status is still not satisfactory [1]. That is why besides the medical doctors and hygienists also the chemists should be involved into the research. They could, after acquiring of a suitable method for the determination, contribute to the knowledge on the supply of iodine to the population.

Moreover, they could cooperate on a program for the improvement of iodine supply by means of food chain, in order to balance the parameters with those in developed countries.

In a majority of the methods used the initial step for the determination of iodine in biological materials includes its conversion into a suitable chemical form, such as iodide or iodate, which may be reliably assayed. Such conversion is achieved either by dry ashing in an alkaline medium in a muffle furnace (600 °C) or wet ashing involving the digestion in a strong acid medium. However, to obtain reproducible results the losses of iodine during the ashing procedure should be avoided. The experiments with radioactive iodine [2] showed that the recovery of iodine with alkaline incineration was still unsatisfactory even when sodium carbonate had been replaced by potassium carbonate [3]. Whatever the procedure used, the digestion is definitely the most critical step in the estimation of iodine.

Following that step, iodine is determined by its reduction action on Ce^{4+} ions coupled to the oxidation of As^{3+} to As^{5+}. The reaction called the Sandell—Kolthoff reaction [4] has been expressed as follows

$$2\,Ce^{4+} + 2\,I^- \longrightarrow 2\,Ce^{3+} + I_2$$
$$2\,Ce^{4+} + 2\,IO_3^- \longrightarrow 2\,Ce^{3+} + I_2 + 3\,O_2$$
$$I_2 + As^{3+} \longrightarrow As^{5+} + 2\,I^-$$

The reduction of Ce^{4+} ions to Ce^{3+} changes the optical absorbance and the progress of the reaction is best monitored by following the decolorization of cerium tetraammonium tetrakis(sulfate). Valuable discussions of the Sandell—Kolthoff reaction have been published in [5, 6]. The role of iodide ions in this reaction is highly specific, since chloride and bromide ions show only slight catalytic activities. The reaction is dependent upon the temperature and the concentrations of sulfuric acid and chloride. They are both important components of the reaction mixture; sulfuric acid increases the reaction rate and chloride stabilizes it by inhibiting the oxidation of iodine to iodate. The reaction mixture has to be kept fairly acidic to prevent the precipitation of

cerium(IV) arsenate. Arsenite is present in large excess.

The most important and easily available biological material for determination of iodine is human urine. Since the content of iodine in urine is a direct indicator of daily iodine consumption, the attention of scientists was focused first of all on the determination of iodine content in this material.

Pineda [7] suggested to proceed directly to the colorimetric assay prior to the digestion or ashing. He found that the iodine concentration determined in the undigested sample was about 72 % of the value found for the same sample when digested and proposed a correction factor to adjust for this difference.

Garry [8] described a method using the dialysis rather than digestion prior to colorimetric determination of urinary iodine and found that dialyzed samples gave essentially the same concentration values as digested ones. In contrast to that, *May et al.* [9] reported that the dialysis technique gave artificially high iodine concentration values for urine samples from iodine-deficient areas of China.

Ion specific electrodes may be used for a determination of iodine in urine, but these are currently not sensitive enough to recognize the low amounts [10].

Neutron activation is highly sensitive and specific, but cannot be used in large number of samples in a routine manner [11].

The aim of this study was to modify the method for iodine determination by using the wet ashing and to verify its validity for the estimation of microquantities of iodine.

EXPERIMENTAL

All chemicals used: nitric acid (conc.), perchloric acid (conc.), arsenic trioxide, sodium chloride, sodium hydroxide, sulfuric acid (conc.), cerium tetraammonium tetrakis(sulfate), deionized water, potassium iodate were of anal. grade.

The following solutions were used: As_2O_3 (5 g) in deionized water (200 cm^3) with 1.75 M-H_2SO_4 (500 cm^3), filled by water to 1 dm^3; $(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$ (3.2 g) in deionized water with conc. H_2SO_4 (80 cm^3), filled by water to 1 dm^3.

Standard iodate solution was prepared by dissolving KIO_3 (0.1685 g) in deionized water to a final volume of 1 dm^3. From this 1 cm^3 is filled by water to 100 cm^3 ($\rho(I^{5+})$ = 1 μg cm^{-3}). From this solution seven standards were prepared by pipetting 15, 25, 37.5, 50, 60, 75, 100 mm^3 and adding deionized water to a final volume of 250 mm^3.

For measurement photocolorimeter Spekol 211 (Zeiss, Jena) with a vacuum suction of the samples and high purity germanium (HPGe) detector for gamma ray measurements of 12.5 % relative efficiency (relative to NaI crystal of ∅ 7.62 × 7.62 cm dimensions) and full width at half maximum (FWHM) 1.92 keV for 1.33 MeV gamma peak of ^{60}Co were used.

Method

250 mm^3 of urine samples (and each standard) were pipetted into 15 cm^3 tubes, 750 mm^3 of the mixture HNO_3—$HClO_4$ (φ_r = 1 : 4) were added and heated for 60 min in thermoblock at 100—120 °C, then cooled to room temperature. 5 cm^3 of As_2O_3 solution were added and mixed. Then 4 cm^3 of 0.005 M ceric solution were added. After 15 min the absorbance of samples and standards at λ = 405 nm was measured using the photocolorimeter.

The temperature, heating time, and cooling time vary within each determination, but the interval between the time of addition of ceric solution and the time of reading must be the same for all samples, standards, and blank.

The facilities for iodine determination must be kept separately from other laboratory activities in order to avoid the contamination and unreproducible results. The use of deionized water and especially of clean chemical glass (all glass must be washed in chromosulfuric acid and three times rinsed by deionized water) is necessary. All chemicals must be free from traces of iodine.

Iodine mass loss after thermal treatment was estimated experimentally using radioactive iodide. From ^{125}I in NaI form (activity 625 kBq cm^{-3}) 18 standards were prepared by pipetting 20, 30, 50, 75, 100, and 125 mm^3 (each volume 3 ×) and adding of deionized water to a final volume of 125 mm^3 into 15 cm^3 tubes. To each tube, 125 mm^3 of stable NaI (amount of carrier 200 mm^3 in 1 dm^3) and 750 mm^3 of the mixture HNO_3—$HClO_4$ (φ_r = 1 : 4) was added. The activity of all samples was measured by HPGe detector before thermal treatment and after that.

RESULTS AND DISCUSSION

The changes of absorbance by reduction effect of KIO_3 standard in the Sandell—Kolthoff reaction can be seen in Fig. 1. The curve *1* represents this interrelation at 15 min after the addition of ceric solution. The curve *2* shows the evolution of the effect of KIO_3 after previous warming with the HNO_3—$HClO_4$ mixture. This curve is the base for the evaluation of the concentration of iodine in the biological material. It is necessary to note that in each individual determination the series of standard dilutions is being treated by the same way and under the same conditions as the samples.

Table 1. The Change of Activity of ^{125}I Standard (in NaI Form) after Thermal Treatment with the HNO_3—$HClO_4$ Mixture

Sample	1	2	3	4	5	6
Activity ^{125}I / kBq	12.5	18.8	31.3	46.6	62.5	78.1
$-\Delta m(^{125}I)$ / %	19.67 ± 2.11	20.20 ± 3.01	21.13 ± 6.06	19.90 ± 3.67	17.13 ± 3.72	20.67 ± 2.99

To each sample 125 mm^3 of stable NaI was added from the stock solution containing 200 μg dm^{-3}.

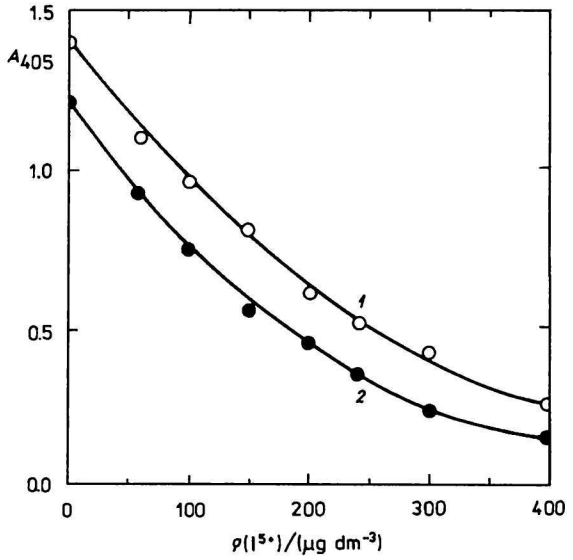

Fig. 1. The change of absorbance for various concentrations of KIO_3 standard in the Sandell—Kolthoff reaction. *1*. Without thermal treatment; *2*. after thermal treatment.

The highest concentration of iodine which may be estimated by this technique is 400 μg dm^{-3}. The samples with higher concentrations of iodine are decolorized and therefore it is necessary to dilute them before the estimation.

We have experimentally determined the volume ratio of the reaction mixture HNO_3—$HClO_4$ to be 1 : 4 and it appeared to be the optimal one for the best mineralization of the samples. When modifying the method we have further used the knowledge of several authors [12, 13] and tried to make this method to be rapid and usable for the routine determination of greater number of samples.

We have tested this method with the samples of human urine and the values of iodine concentration varied up to 200 μg dm^{-3}. The method can be used also for the determination of iodine content in food, but it is necessary to take the optimal quantity of material and to prolong the time of digestion up to the total mineralization of the samples.

The highest losses of iodine were found at dry or wet ashing. Actually, this is the step resulting in relatively most considerable errors when compared with the other much less critical steps. The iodine losses by our way of mineralization were checked with the aid of radioactive iodine. The changes of various concentrations of ^{125}I (with carrier NaI) were measured before and after thermal processing (for 60 min at 100—120 °C). The results may be seen in Table 1.

The pooled mean value of iodine mass losses which was obtained from 18 estimations was (19.78 ± 0.92) % at the 95 % confidence level. Because the losses are not too high, the used technique is suitable for the determination of microquantities of iodine. The described method of iodine determination in biological material is a micromethod suitable for the determination of iodine concentration within the range of 10—400 μg dm^{-3}.

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