

2. On oxyammonolysis, cellolignin forms approximately twice as much water-soluble fraction as hydrolysis lignin, while lignosulfonate is desulfonated under conditions above the optimum.

3. The maximum nitrogen content of the water-insoluble fraction of lignin (nitrogen-containing lignin) amounts to 9.0% for cellolignin and is 2-3% less for the others.

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DEVELOPMENT AND COMPARATIVE EVALUATION OF METHODS FOR THE MICRODETERMINATION OF IODINE IN DRUGS OF NATURAL ORIGIN

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546.15:58.08

Investigations have been made with the aim of drawing up a new and all-sided comparative evaluation of various known methods for the microdetermination of iodine in order to select the optimum procedures for the quantitative analysis of this element in materials of animal and plant origin. The efficacy of the use of the method of combustion in a flask with oxygen in the analysis of thyroidin and laminaria has been shown, and a new procedure for the microdetermination of iodine based on the combustion of a sample in oxygen and the determination of the iodine formed photometrically and by the starch-iodide reaction has been proposed.

Iodine-containing drugs of natural origin (thyroidin — a powder of the thyroid glands of animals, and laminaria — sea kale) have long been widely used in medical practice. Their therapeutic effect is due in part, to the presence of iodine [1]. Therefore, in the standardization of such raw material for medical purposes and also its prepared medicinal forms and in biochemical investigations various methods for the quantitative determination of this element are used that are based on the employment of several variants of the mineralization of the sample [2, 3], iodine-selective electrodes, differential pulsating polarography, kinetic methods, etc. [4, 5]. However, the determination of iodine in such specific material is a complex analytical problem. This is due to the small amount of iodine in materials of natural origin, the complexity of their chemical compositions, the volatility of iodine, and the possibility of its passage during mineralization into compounds with different degrees of oxidation.

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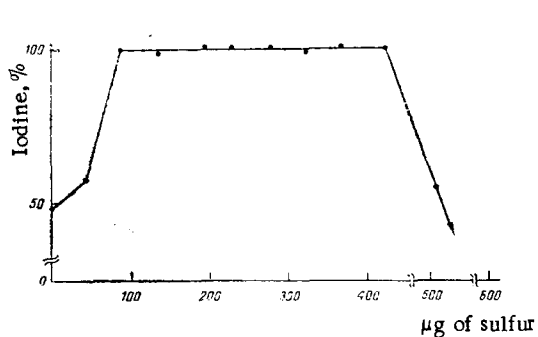


Fig. 1

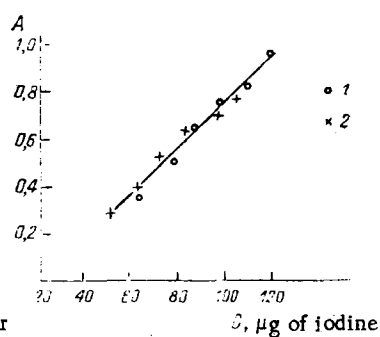


Fig. 2

Fig. 1. Graph of the dependence of the amount of iodine found on the sulfur content of the sample (for explanation, see text).

Fig. 2. Graph of the dependence of the optical density of the starch-iodine complex on the amount of iodine (for explanation, see text): 1) for a diiodotyrosine standard; 2) for a solution of potassium iodide.

In this connection, in the practical respect the choice of a method permitting the microdetermination of iodine in natural samples with adequate reliability and accuracy is important. In the present paper we give results of investigations with the aim of developing new procedures for the microdetermination of iodine and making an all-sided comparison of various known procedures in order to select the optimum method for the quantitative analysis of this element in materials of animal and vegetable origin. For this purpose we used known methods (especially pharmacopoeal methods) and methods that we have developed previously for the analysis of thyroidin and laminaria based on the use of "dry" or "wet" mineralization and combustion in oxygen [2, 3, 6-13].

In addition, with the aim of developing faster and simpler methods for the microdetermination of iodine and broadening their extremely limited variety, we have proposed a new method for the quantitative determination of this element using photometry and combustion in a flask with oxygen which has been recognized with the grant of an Inventors' Certificate [14]. The use of the latter method in the performance of these investigations was due to its obvious advantages, consisting in the rapid (in the course of a few seconds) mineralization of the sample under investigation by combustion in a closed flask with oxygen followed by the sensitive express photometric determination of the iodine formed, and also by the exceptional simplicity of the apparatus required.

The determination of iodine using combustion in a flask of oxygen and photometry is connected with the choice of a sensitive and specific reaction for determining the iodine after the combustion of the sample and the absorption of its combustion products. With this aim we used the starch-iodine reaction as that corresponding most closely to the above-mentioned demands. The use of starch is extremely attractive because it possesses a high adsorption capacity in relation to iodine. This permits an investigation of the possibility of using it simultaneously also as a highly effective absorbing solution in the oxygen-flask combustion method. We showed the possibility of such use of a solution of starch previously for the qualitative determination of iodine in thyroidin [15] and the quantitative analysis of highly iodinated compounds by combustion in oxygen [16]. Then we also established the necessity for the presence in the starch solution of sulfamic acid in order to decompose the excess of nitrite ions formed in the combustion of natural samples, which interfere with the starch-iodine reaction [15]. Consequently, in quantitative analysis we used a similar (0.2%) concentration of sulfamic acid in the absorbing solution (a 0.5% aqueous solution of starch).

The most important problem in the development of this method was the study of the conditions for the stoichiometric formation of molecular iodine on the combustion of iodine-containing compounds in a flask with oxygen, and also its quantitative absorption and determination in the starch solution. For this purpose, it was necessary to compare the calibration graph for photometric determination plotted with the use of a standard solution of inorganic iodine (potassium iodide) with the analogous calibration graph obtained as the result of the

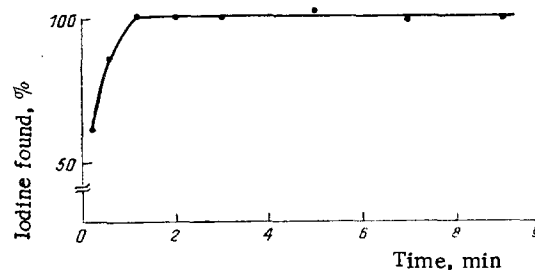


Fig. 3. Dependence of the amount of adsorbed iodine on the time. The amount of iodine in thyroïdin found previously by other methods [7, 8] was taken as 100%.

TABLE 1. Results of the Quantitative Determination of Iodine

Sam- ple*	Iodine taken, mg	Optical density	Iodine found		Metrological characteristics
			mg	%	
I	0,053	0,29	0,054	101,89	$\bar{x}=99,81$
	0,063	0,36	0,062	98,41	$S=1,64$
	0,074	0,44	0,072	97,30	$S_x=0,62$
	0,084	0,55	0,085	101,19	$E_{0,95}=1,52$
	0,095	0,64	0,095	100,00	$A_{rel}=\pm 1,52\%$
	0,105	0,72	0,104	99,05	
II	0,116	0,83	0,117	100,86	
	0,0384	0,52	0,081	0,211	$\bar{x}=0,216$
	0,0391	0,54	0,084	0,215	$S=6,34 \cdot 10^{-3}$
	0,0383	0,56	0,086	0,225	$S_x=2,40 \cdot 10^{-3}$
	0,0386	0,56	0,086	0,223	$E_{0,95}=5,87 \cdot 10^{-3}$
	0,0408	0,59	0,089	0,218	$A_{rel}=\pm 2,72\%$
III	0,0357	0,46	0,074	0,207	
	0,0375	0,52	0,081	0,216	
	0,0226	0,38	0,065	0,324	$\bar{x}=0,324$
	0,0215	0,38	0,065	0,341	$S=15,02 \cdot 10^{-3}$
	0,0287	0,50	0,078	0,306	$S_x=6,72 \cdot 10^{-3}$
	0,0284	0,56	0,085	0,337	$E_{0,95}=18,65 \cdot 10^{-3}$
	0,0285	0,50	0,079	0,313	$A_{rel}=\pm 5,76\%$

*I) Model sample; II) thyroid powder; III) sugar blade kelp

combustion of various amounts of a standard organic compound with a known iodine content. The coincidence of such graphs would indicate the possibility of the quantitative determination of iodine in organic substances by the proposed method.

The results obtained in the combustion of the standard organoiodine compound proved, in a number of cases, to be 10-15% lower than the results obtained with the use of the standard iodide solution. At the same time, the combustion of a weighed amount of thyroïdin powder (about 40 mg) indicated the quantitative determination of the iodine in it with the production of results close to those obtained by the use of other methods of analyzing thyroïdin [7-13]. As we have mentioned previously, the elementary analysis of thyroïdin shows the presence of nitrogen and sulfur, which promote the appearance of the molecular form of iodine after the combustion or organoiodine samples [15].

In order to make a detailed study of the influence of the elementary composition of the substance undergoing combustion on the quantitative determination of iodine, we analyzed standard samples of diiodotyrosine with the addition to it of organic compounds containing nitrogen and sulfur.

The results of the investigation are shown in Fig. 1. As follows from these results, the quantitative determination of about 80 μg of iodine requires the presence of 65-400 μg of sulfur in the sample. Smaller or larger amounts of sulfur lead to low results. Roughly similar

TABLE 2. Determination of Iodine in Whole and Ground Thyroidin Tablets

Tablets	Results of analysis of the tablets	
	ground	whole
0.05 g each	$n = 5$ $\bar{x} = 0.117$ $S = 4.42 \cdot 10^{-3}$ $S_{\bar{x}} = 1.98 \cdot 10^{-3}$ $E_{0.95} = 5.50 \cdot 10^{-3}$ $A_{rel} = \pm 4.46\%$	$n = 5$ $\bar{x} = 0.117$
0.1 g each	$n = 5$ $\bar{x} = 0.218$ $S = 8.57 \cdot 10^{-3}$ $S_{\bar{x}} = 3.83 \cdot 10^{-3}$ $E_{0.95} = 10.63 \cdot 10^{-3}$ $A_{rel} = \pm 4.38\%$	$n = 5$ $\bar{x} = 0.223$

The analysis of whole tablets does not permit the metrological characteristics of the procedure used to be established objectively, since the discrepancies in the individual results are due not only to the error of analysis but also to a certain divergence in the amounts of iodine in the individual tablets. The metrological characteristics of the analytical procedures developed were therefore determined in the analysis of a mixture of ground tablets.

results were obtained in an analysis of samples containing 50, 120, and 230 μg of iodine. We may note that the influence of nitrogen is less pronounced. The investigation showed that its presence in the model samples investigated within wide limits, from 0.1 to 6 mg, had no influence on the quantitative determination of iodine.

The results obtained confirmed earlier work [15] on the influence of the elementary composition of thyroidin on the formation of molecular iodine and its determination by the starch-iodine reaction.

A calibration graph plotted as the result of the analysis of various amounts of diiodo-tyrosine when the samples also contained 150-200 μg of sulfur (as thiourea) and 5 mg of nitrogen (as aminoacetic acid) agreed with the calibration curve obtained by the use of a standard solution of iodine (Fig. 2).

Under these conditions of determination, Beer's law was observed at iodine concentrations of between 50 and 120 μg in 5 ml of absorbing solution. At lower concentrations of iodine (the limit of detection of which is about 35-40 μg), the optical density of the solution was unstable and depended to a considerable degree on the temperature.

The spectra in the visible region of the starch-iodine complex that were obtained (see the Experimental part) in the plotting of the calibration graph and after the combustion of thyroidin tablets coincided, having an absorption maximum at 590 nm.

The subsequent investigations were devoted to a study of the influence of the volume of the flask and the time of absorption on the quantitative determination of iodine. The results,

TABLE 3. Determination of the Homogeneity of Thyroidin in Tablets (on the basis of two series)

Tablets	No. of the analysis	Optical density	Iodine found in a tablet, mg	Deviation from nominal	
				mg	%
0.05 g each, series 30379	1	0,56	0,086	-0,014	-14
	2	0,57	0,087	-0,013	-13
	3	0,55	0,085	-0,015	-15
	4	0,56	0,086	-0,014	-14
	5	0,49	0,078	-0,022	-22
	6	0,54	0,083	-0,017	-17
	7	0,48	0,077	-0,023	-23
	8	0,43	0,071	-0,029	-29
	9	0,49	0,078	-0,022	-22
	10	0,56	0,086	-0,014	-14
0.1 g each, series 70379	1	0,45	0,218	+0,018	+9
	2	0,46	0,224	+0,024	+12
	3	0,42	0,209	+0,009	+4,5
	4	0,45	0,218	+0,018	+9
	5	0,44	0,215	+0,015	+7,5
	6	0,38	0,194	+0,006	+3
	7	0,40	0,201	+0,001	+0,5
	8	0,40	0,201	+0,001	+0,5
	9	0,51	0,242	+0,042	+21
	10	0,37	0,191	-0,009	-4,5

which are given in Fig. 3, show that the completeness of the absorption of iodine in flasks with a volume of 1 liter can be achieved in only 1 minute after the combustion of the sample under investigation. Identical results were obtained on the combustion of the same samples in flasks with volumes of 350 and 500 ml. We may also note that the positioning of the holder with the analytical sample in the flask for combustion at a distance of 1-2 cm from the absorbing solution led to an acceleration of the absorption process.

The results obtained indicate a high efficacy of the use of starch in the absorbing solution (for comparison: according to [17] not less than 30 min is required for the absorption of iodine by alkali).

On the basis of the investigations performed, procedures have been proposed for the quantitative determination of iodine in samples of natural origin and medicinal preparations of them which have been subjected to preliminary testing in model experiments. The results of the analyses are given in Table 1.

In the analysis of tablets, iodine was determined in each individual tablet by its combustion in the integral state, without grinding. Such determinations permit the study of the uniformity of the dosing of drugs in individual doses, to which increased attention has been recently devoted [18, 19]. The results in Table 2, illustrating a comparison of the results from the analysis of whole and ground tablets, confirm the possibility established previously [9] of the combustion and analysis of tablets without their preliminary grinding.

The results of the analysis of the tablets (Table 3) showed a nonuniformity of the dosage of thyroidin in the tablets. In a number of cases we have obtained similar results previously with the use of a different procedure for the quantitative determination of iodine [9].

We may note that in the analysis of thyroidin the addition of organosulfur compounds need not be used, since sulfur is a component of the protein thyroglobulin (its content is about 0.4% [15]).

The results of a comparative evaluation of various procedures for the microdetermination of iodine performed by using them for the analysis of identical samples of thyroidin and laminaria (Table 4) confirmed that one of the procedures gave high results in the analysis of thyroidin, as was previously reported [8]. The other procedures permit results in good agreement to be obtained.

Thus, the most suitable procedures from the point of view of rapidity, sensitivity, and unification are those based on the use of the method of combustion in a flask with oxygen (including the new method using the final photometric determination of iodine).

TABLE 4. Comparative Evaluation of Methods of Determining Iodine in Thyroidin and Laminaria

Sample	Procedure	Metrological characteristics			
		n	\bar{x}	s	$A_{rel}, \%$
I. Thyroidin powder	1. British pharmacopoeia [12] (combustion in oxygen)	7	0,312	$12,9 \cdot 10^{-3}$	$\pm 3,8$
	2. United States Pharmacopoeia [11] ("dry" mineralization)	5	0,252	$3,0 \cdot 10^{-3}$	$\pm 1,6$
	3. Combustion in oxygen on platinum (titrimetric determination) [8]	7	0,254	$3,0 \cdot 10^{-3}$	$\pm 1,1$
	4. Combustion in oxygen on nichrome (titrimetric determination) [8]	7	0,249	$7,4 \cdot 10^{-3}$	$\pm 2,7$
	5. Combustion in oxygen on nichrome (photometric determination)	7	0,249	$10,0 \cdot 10^{-3}$	$\pm 3,7$
	6. "Dry" mineralization [7]	5	0,251	$2,5 \cdot 10^{-3}$	$\pm 0,4$
II. Sugar blade kelp	1. FS № 42-1289-79	5	0,333	$63,3 \cdot 10^{-3}$	$\pm 23,6$
	2. Combustion in oxygen on nichrome (titrimetric determination) [6]	5	0,308	$6,4 \cdot 10^{-3}$	$\pm 2,6$
	3. Combustion in oxygen on nichrome (photometric determination)	5	0,324	$15,0 \cdot 10^{-3}$	$\pm 5,8$

In addition to the results given in this paper on the photometric determination of iodine, the table makes use of other methods of analyzing thyroidin and laminaria that we have published previously [6-8].

EXPERIMENTAL

In the method of combustion in a flask with oxygen we used an instrument that we have described previously [20]. The technique of combustion has been described in [21, 22].

Reagents Used. A standard solution of potassium iodide was obtained by dissolving 0.07 g (accurately weighed) of potassium iodide (kh.ch ["chemically pure"]) in water in a 500-ml measuring flask.

A standard solution of diiodotyrosine was obtained by dissolving 0.5 g (accurately weighed) of pure diiodotyrosine (Merck standard for elementary analysis) in 0.1 N sodium hydroxide in a 500-ml measuring flask.

A standard solution of thiourea was prepared by dissolving 1.5 g (accurately weighed) of thiourea (chemically pure) in water in a 500-ml measuring flask. The absorbing reagent was obtained by dissolving 0.2 g of sulfamic acid (chemically pure) in 100 ml of a 0.5% solution of soluble starch (GOST [State Standard] 101163-76).

The glycine (aminoacetic acid) was a Reanal (Hungary) standard.

The other substances and reagents have been given in [2].

The plotting of a calibration graph with respect to the standard iodine solution was based on the determination of the molecular iodine formed from potassium iodide under the action of nitrite ions followed by the photometry of the starch-iodine complex. The procedure was based on that given in a monograph [23]. The calibration curve obtained is shown in Fig. 2.

Study of the Influence of Nitrogen and Sulfur on the Quantitative Determination of Iodine in Organic Substances. Various amounts (15-20 μ l) of the standard solution of diiodotyrosine were deposited with the aid of a microsyringe on pieces (1 \times 1 cm) of ash-free filter paper folded in the form of a cone and fixed to the holder of the instrument for combustion.

With the aid of a microsyringe, various amounts of a 3% solution of the thiourea and 15-30 mg of glycine were added to these samples. The samples were burnt in 250-ml flasks with oxygen. The iodine was absorbed in 5 ml of absorbing reagent, the time of absorption being 10 min. Then the solutions were photometered on a FEK-56 PM instrument with a No. 7 filter (transmission maximum at 582 nm) in cells with an optical layer 5 mm thick at 19-20°C. The choice of filter was determined by the absorption spectrum of the starch-iodine complex, which has a maximum at 590 nm. The absorbing solution was used as the comparison solution. The amount of absorbed iodine was determined from a calibration curve plotted by the use of the standard solution of potassium iodide or of diiodotyrosine.

Investigation of the Influence of the Volume of the Flask and the Time of Absorption on the Quantitative Determination of Iodine. Model samples containing diiodotyrosine (about 0.1 mg of iodine), thiourea (180 µg of sulfur), and glycine (5 mg of nitrogen) were analyzed as described above.

To study the time of absorption, combustion was performed in 250-ml flasks and, in the investigation of the influence of the volume of the flask, flasks with volumes of 250-1000 ml were used.

The Plotting of a Graph with a Standard Solution of Diiodotyrosine and Determination of Organically Bound Iodine in Model Experiments. Various amounts of a standard solution of diiodotyrosine (50-250 µg of iodine) were analyzed with the addition of 15 µl of a standard solution of thiourea (150-200 µg of sulfur) and 25 mg of aminoacetic acid (5 mg of nitrogen). The determination was performed as described below in the analysis of thyroidin.

Quantitative Determination of Iodine in Thyroidin. About 0.03-0.05 g of sample (an accurately weighed amount of thyroidin containing 50-120 µg of organic iodine) was transferred to a 20 × 20 mm piece of cellophane folded in the form of a packet and fixed to the support of the instrument for combustion. The 250- to 400-ml combustion flask was charged with 5 ml of absorbing solution so that the inner walls of the flask were wetted with it. The sample was burnt, and after 30 sec the contents of the flask were shaken for 3 min with the simultaneous cooling of the flask in a vessel with cold water having a temperature of 10-15°C. Then the absorbing reagent was filtered through a small piece of adsorbent cotton into a cell with a thickness of the absorbing layer of 5 mm and was photometered on a FEK-56 instrument (filter No. 7) at 19-20°C. The absorbing solution was used as the comparison solution. The amount of iodine was found from a calibration graph plotted as described previously (using a solution of potassium iodide or standard diiodotyrosine).

Quantitative Determination of Iodine in 0.05-g Thyroidin Tablets. One tablet of the preparation wrapped in a narrow strip of ash-free paper with dimensions of 2 × 30 mm was placed in the holder of the instrument for combustion and was then analyzed as in the analysis of the thyroidin powder.

Quantitative Determination of Iodine in 0.1-g Thyroidin Tablets. The analysis was performed similarly. A flask with a volume of 500-600 ml was used for combustion, and 15 ml of the absorbing reagent for absorption. The iodine content was calculated from a calibration graph as in the analysis of thyroidin powder but with allowance for the threefold dilution of the solution under investigation.

Quantitative Determination of Iodine in Laminaria. About 0.03 g of sample (an accurately weighed powder of sea kale ground as shown in FS [Pharmaceutical Specification] 42-1289-79) was placed on a piece of cellophane or of ash-free filter paper with dimensions of 15 × 15 mm upon which 20 µl of the standard solution of thiourea had previously been deposited. The further procedure was as in the analysis of thyroidin powder.

SUMMARY

1. A new rapid method for the microdetermination of iodine in samples of natural origin has been developed which is based on the combustion of the sample in a flask with oxygen and the photometric determination of the iodine formed by means of the starch-iodine reaction.

2. A comparative evaluation has been made of the various methods for the microdetermination of iodine in samples of animal and vegetable origin which indicates the efficacy of the method of combustion in a flask with oxygen.

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