Spectrophotometric and iodometric methods for the detection of hydrogen sulfide in the Black Sea: comparison of the results of analysis*

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Abstract — We discuss the results of analysis of published data and field and laboratory investigations aimed at the solution of the problem of comparability of the results of detection of hydrogen sulfide in the Black Sea by spectrophotometric and iodometric methods. We show that the reproducibility of the results of spectrophotometric analysis is higher than hat of the iodometric method only in the case where the content of sulfide in a sample is less than 10–30 μM l^{-1}. When larger concentrations are involved, the traditional iodometric analysis proves to be the most precise and reliable method for the detection of hydrogen sulfide in the Black-Sea waters.

INTRODUCTION

The detection of hydrogen sulfide is of exceptional importance for the analysis of the state of the Black-Sea ecosystem and the prognosis of its development because the presence of this substance beginning with 80–250-m depth [1] rules out the possibility of functioning of the usual biological communities. Since hydrogen sulfide and oxygen intensively interact, there exists a transition zone in the Black-Sea waters [1–4] in which the concentration of both substances is small. Low contents, as well as the great interest in the investigations of the hydrochemical structure of the transition zone [2, 3, 5], determine a special attention given to analytical methods for the detection of oxygen [6, 7] and hydrogen sulfide [7–9]. At present, three qualitatively different and more or less widespread methods for the detection of hydrogen sulfide in the Black-Sea waters are known, namely, the iodometric method (IDM) [10], the spectrophotometric method (SPM) [11], and the polarographic method [12].

Traditionally [1, 10, 13], for the detection of hydrogen sulfide in the Black-Sea waters, one used the method of reverse titration of the excess of iodine left after the interaction with a sample by a solution of sodium thiosulfate (the iodometric method). The overwhelming majority of results were obtained precisely by this method. In this case, the term "hydrogen sulfide" is understood as the sum of both hydrogen sulfide (H_2S) dissolved in water together with the products of its dissociation (HS^-, S^{2-}) and the reduced compounds of sulfur (colloid sulfur (S°), sulfites (SO_3^{2-}), polysulfides (S_x^{2-}) and polythionates ($S_2O_3^{2-}$, $S_4O_6^{2-}$) [1, 2, 14]). This sum also includes an insignificant quantity of organic compounds dissolved and sus-

pended in seawater, expressed in the equivalent amount of hydrogen sulfide. The precision of the detection of the sum of reduced compounds by the method of reverse titration significantly depends on the precision of titration and selection of a water sample for the determination of the ratio of concentrations of iodine solution and sodium thiosulfate [8, 9].

The spectrophotometric method [11, 15] was less frequently used for the detection of hydrogen sulfide in the Black-Sea waters [3, 14, 16–18]. An apparent reason for this lies in the fact that, at the initial stage, necessary reagents and equipment were hardly accessible; later, there arose the question of possibility and correctness of comparison of data [1, 19–21] obtained by different methods.

According to refs 11 and 12, the spectrophotometric method, which consists of measuring the optical density of methylene blue formed by adding a mixture of solutions of salines of N, N-dimethyl-para-phenylenediamine and ferric iron to a sample under analysis, allows one to selectively determine the content of hydrogen sulfide and its dissociation products (H_2S, HS^-, and S^{2-}) in the presence of other reduced compounds of sulfur. According to ref. 11, the precision of this method for the detection of hydrogen sulfide in a standard solution is ±2% with 95% confidence level. However, to our knowledge, experimental data that confirm the selectivity of the spectrophotometric method and its precision for samples of real seawater are absent in the literature.

Moreover, there exists a certain disagreement between the results of comparison of the spectrophotometric and iodometric methods for the detection of hydrogen sulfide published by different authors [1–3, 19]. Thus, in ref. 19, the coincidence of the results of the spectrophotometric and iodometric analyses of estuary waters with hydrogen-sulfide concentration of 160–4530 μmole liter^{-1} was reported. A certain correspondence between the results obtained by both methods under discussion was also noted in ref. 1. In ref. 2, on the basis of analysis of data published in refs 3 and 14, the following conclusion was drawn: "... the photometric method for the detection of hydrogen sulfide agrees with the iodometric method only for concentrations of hydrogen sulfide below 0.6–0.8 ml liter^{-1} (27–36 μmole liter^{-1}). If larger concentrations are involved, the photometric results become noticeably higher (!) than the iodometric ones, which is reflected in the variation of the gradient of hydrogen-sulfide concentration. This must be taken into account in comparing data obtained for deeper waters by different methods." At the same time, in ref. 3, it was proposed to use the spectrophotometric method for the detection of hydrogen sulfide at concentrations that do not exceed 1–1.5 ml liter^{-1} (~ 45–60 μmole liter^{-1}) without diluting samples, and it was noted that, for larger concentrations of hydrogen sulfide, the understatement (!) of results as compared with the iodometric method takes place. In ref. 20, it was reported that the results of determination of the location of the boundary of anaerobic waters by the iodometric and spectrophotometric methods showed no difference. According to ref. 20, the analysis of 57 samples from the upper part of the anaerobic zone (the concentration of hydrogen sulfide was 0–45 μmole liter^{-1}) by these two methods showed good agreement between their results. Certain differences were detected in deeper waters, and it was stated in [20]

that these differences were compensated by the presence of thiosulfates. However, Fig. 1, plotted according to the data of ref. 20, shows that, for the entire range of concentrations of hydrogen sulfide, there was a systematic understatement of the spectrophotometric results with respect to those of the iodometric method, on average, by 25% and that, for individual samples, this difference reached 50%. Such a significant difference in the results, especially when large concentrations of hydrogen sulfide are involved (20 μmole liter^{-1}), cannot be explained by the presence of thiosulfates or other reduced compounds of sulfur. Probably, it can be explained by the peculiarities of the used modification of the spectrophotometric method or insufficient purity of N, N-dimethyl-para-phenylenediamine.

Thus, in the scientific literature, there is no unique answer to the question of comparability of data obtained by the iodometric and spectrophotometric analyses even on the qualitative level. For this reason, the aim of the present work was to carry out a comparative investigation of the iodometric and spectrophotometric methods in laboratory and field conditions. We also posed the problem of quantitative comparison of the data obtained by these two methods, development of a basis for comparison of available data, testing of the selectivity of the spectrophotometric method, and investigation of the influence of the products of oxidation of hydrogen sulfide on the results.
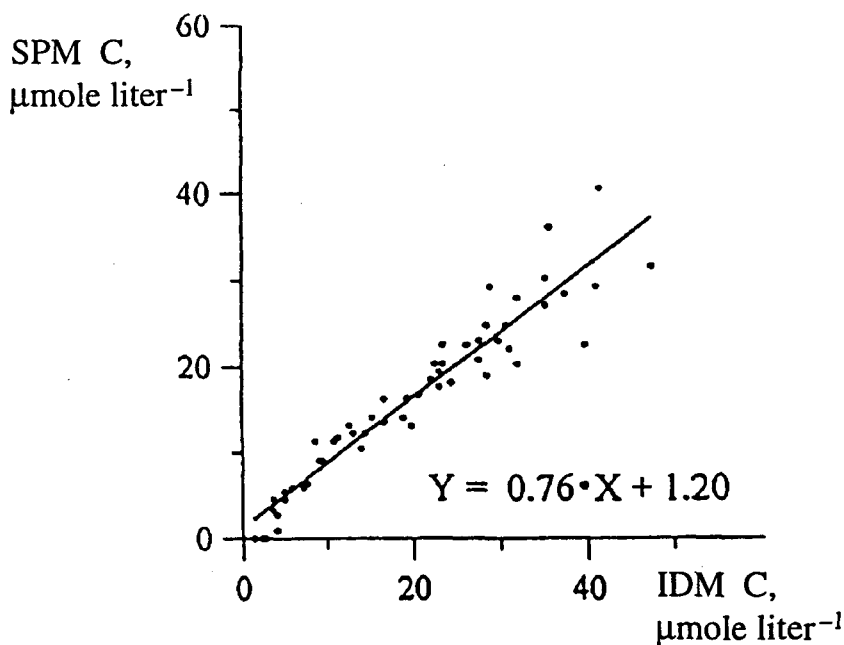

Figure 1. The results of comparison of the parallel detection of hydrogen sulfide by the spectrophotometric and iodometric methods on the basis of the data of ref. 20.
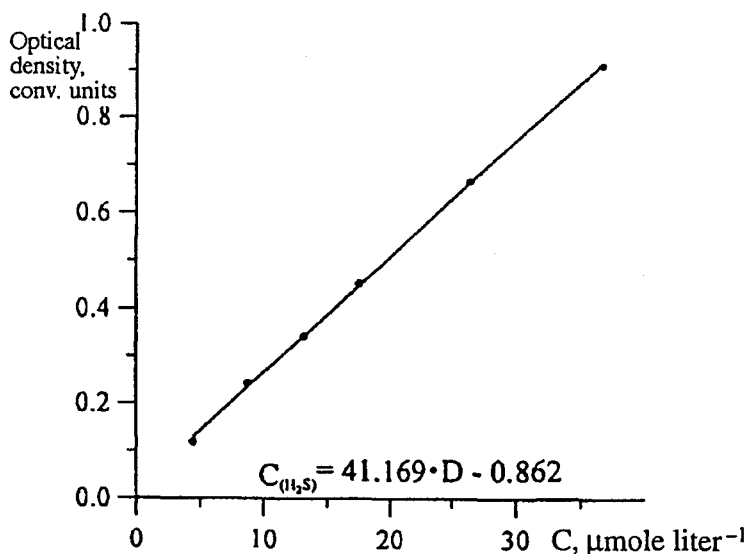
Figure 2. Calibration curve of the spectrophotometric detection of hydrogen sulfide.

MATERIALS AND METHODS

In this work, we used the laboratory and field data of the iodometric [7] and spectro-photometric [11] analysis of hydrogen sulfide in the Black-Sea waters and in pre-pared solutions containing sodium sulfide. It should be noted that, in the iodometric analysis of the Black-Sea water, the ratio of iodine solution and thiosulfate was de-termined as the average value for the samples from the levels located directly above the boundary of the spread of hydrogen sulfide (as a rule, these were the depths of the isopycnic surfaces 15.8–16.0). In methodological guides accepted for the use in the systems of scientific [23] and hydrometeorological [24] institutions, it is recom-mended to use for this purpose the sea-surface water, which can differ from the water of lower layers of the aerobic zone in the content of iodine consumers.

According to ref. 11, for the spectrophotometric analysis one uses reagents of different concentration, depending on the content of sulfides in a sample (see Fig. 1). In the present work, for the detection of hydrogen sulfide with concentration below 40 μmole liter^{-1}, we used solutions corresponding to range No. 2 (see Fig. 1). In the analysis of samples with concentration of hydrogen sulfide below 3 μmole liter^{-1}, this leads, according to ref. 14, to overconsumption of reagents but does not affect the final results.

Determining the content of hydrogen sulfide in the range 40–250 μmole liter^{-1}, we added reagents as indicated in ref. 11. After the development of color, these samples were diluted (1:10) by distilled water.

Table 1.

Concentration of reagents, dilution, and cuvette lengths recommended for various ranges of concentration of sulfides [11]

sulfides (μmole liter^{-1})	Concentration of diamine reagent (g per 500 ml)	chloric iron (g per 500 ml)	Dilution (ml : ml)	Cuvette length (cm)
1–3	0.5	0.75	1:1	10
3–40	2.0	3.0	1:1	1
40–250	8.0	12.0	2:25	1
250–1000	20.0	30.0	1:50	1

Dark glass-stoppered bottles of volume 40 ml and 25-ml graduated cylinders were used for the selection of samples for the spectrophotometric analysis. Both bottles and cylinders were dried and blown off by argon before the selection of samples. In using 40-ml bottles, we applied the same method for sample selection as in the detection of oxygen, i.e., overflow of 1–1.5 volumes of a bottle. Sulfide solutions were prepared in 100-ml Nessler tubes by inserting aliquots of the working solution with subsequent increase in volume to the marker by adding oxygen-free distilled water or seawater. Immediately after selection, we added the reagents and mixed the samples. The measurement of optical density was carried out after 20 min on a concentration photocolorimeter (KFK-3) with wavelength of 670 nm. We used a fresh solution of sodium sulfide for the construction of calibration curves. The exact concentration of sulfide in the working solution was determined by the iodometric method as indicated in ref. 11. A sample calibration curve is presented in Fig. 2.

ANALYSIS OF THE RESULTS

Reproducibility of the methods. The reproducibility of the iodometric method was estimated according to the results of detection of hydrogen sulfide in the samples selected at the same level by the bathometers of the cartridge of a probing complex. The data obtained are presented in Table 2. One can see that the absolute error of detection of hydrogen sulfide is close to 1 μmole liter^{-1}. As a result, the relative error is small for large concentrations of hydrogen sulfide and substantially increases as its content in a sample becomes less than 10 μmole liter^{-1}.

The reproducibility of the spectrophotometric method was estimated according to the results of analysis of prepared solutions of sodium sulfide in distilled water and seawater and analysis of natural samples of Black-Sea water from various depths (see Table 2). In selecting samples of all types, we used bottles of three types, namely, 40-ml brown glass-stoppered bottles, gum-stoppered test tubes with markers at 25 ml, and Nessler glass-stoppered tubes with markers at 100 ml. Doses

of a solution of the mixture of a diamine reagent (N, N-dimethyl-para-phenylenedi-amine) and chloric iron were added according to the volumes of samples.

One can see that, for hydrogen-sulfide concentrations in the range 1.5–95.5 μmole liter^{-1}, the coefficient of variation varied from 2.2 to 9.5% regardless of the type of the original sample, its sulfide content, vessels, and methods for sample selection.

The influence of pH oscillations in samples on the development of sample coloring was tested during Cruise 33 of R/V *Professor Kolesnikov*. It turned out that the differences in pH corresponding to real oscillations of this parameter for samples from the upper part of the hydrogen-sulfide zone (of order 0.03 pH) did not affect the results of the spectrophotometric analysis.

Thus, the data obtained shows that the spectrophotometric analysis enables one to obtain more reproducible data as compared with the iodometric method only for hydrogen-sulfide concentrations in samples below ~10 μmole liter^{-1}. For large contents of hydrogen sulfide, the iodometric analysis proves to be a more reliable method for the investigation of the anaerobic zone of the Black Sea. In this connection, one can propose to simultaneously use the spectrophotometric and iodometric analyses in field conditions; in this case, however, the question about the possibility and correctness of comparison of the results of these methods becomes especially important.

Table 2.

Reproducibility of the results obtained by the iodometric and spectrophotometric methods

Type of a sample	Number of parallel detections	Mean value (μmole liter^{-1})	Standard deviation (μmole liter^{-1})	Coefficient of variation (%)	Volume of a sample (ml)
		Iodometric method			
A	13	1.9	1.3	68	250
A	15	39.0	0.8	2.1	250
		Spectrophotometric method			
A	16	1.5	0.11	7.1	25
A	15	1.8	0.17	9.5	40
A	10	3.2	0.16	5.0	25
A	8	5.9	0.32	5.5	40
B	8	2.2	0.17	7.7	40
B	4	12.2	0.26	2.2	100'
B	3	12.3	0.63	5.1	100
B	4	12.8	0.63	5.0	100
B	5	25.0	0.56	2.2	100
B	8	40.6	1.4	3.4	40
C	8	64.1	3.38	5.3	100
C·	8	95.5	6.94	7.3	100

Note: A denotes seawater, B denotes a solution of sodium sulfide in distilled water, and C denotes a solution of sodium sulfide in seawater.

Table 3.

Content of sulfides at isopycnic surfaces σ_t = 16.30 and 16.50 according to the data of various expeditions

Conv. density	Spectrophotometric method				Iodometric method			
	Mean value $\left(\dfrac{\mu mole}{liter}\right)$	Mean-square deviation $\left(\dfrac{\mu mole}{liter}\right)$	Number of measurements, n	C_{var} (%)	Mean value $\left(\dfrac{\mu mole}{liter}\right)$	Mean-square deviation $\left(\dfrac{\mu mole}{liter}\right)$	Number of measurements, n	C_{var} (%)
			R/V *Knorr*, 1988 [25]					
σ_t = 16.30	8.8	1.8	21	21	–	–	–	–
σ_t = 16.50	29.2	1.9	21	7.0	–	–	–	–
			R/V *Professor Kolesnikov*, 1995					
σ_t = 16.30	8.5	1.4	17	17	14.9	1.7	16	12
σ_t = 16.50	28.5	1.6	12	5.7	28.5	1.6	13	5.7

Comparison of the methods using samples of seawater. In Table 3, we present the data of detection of hydrogen sulfide by two different methods in expeditions of R/V *Knorr* (May–July, 1988) [25] and R/V *Professor Kolesnikov* (March, 1995).

The selected isopycnic surfaces σ_t = 16.30 and σ_t = 16.50 in fact represent narrow intervals of values lying near the mean values within the limits of 0.01 of conventional density unit.

As follows from Table 3, the mean values of hydrogen-sulfide concentrations determined by the spectrophotometric method coincide both for σ_t = 16.30 and σ_t = 16.50, despite the 7-year interval between the expeditions. However, comparison of the results of detection of hydrogen sulfide by the spectrophotometric and iodometric methods shows that the coincidence of results is observed only for σ_t = 16.50 (the content of hydrogen sulfide is 28.5 μmole liter^{-1}. For σ_t = 16.30, the results obtained by spectrophotometric method are almost half the results obtained by the iodometric method (8.5 and 14.9 μmole liter^{-1}, respectively).

Thus, the results of the present work allow us to draw a conclusion about the possibility of comparison of the data obtained by the spectrophotometric method in different expeditions if the same modification of the method was applied, e.g., the one presented in ref. 11. At the same time, comparison of the results obtained by different methods (the spectrophotometric and iodometric ones) reveals ambiguity even for one expedition. Thus, e.g., the larger value of hydrogen-sulfide concentration at σ_t = 16.30 recorded by the iodometric method (Table 3) can be explained by the presence of other forms of reduced compounds of sulfur that are different from hydrogen sulfide and products of its hydrolysis. However, according to ref. 12, the

products of intermediate oxidation of hydrogen sulfide, except for elementary sulfur, are absent in the Black-Sea waters in any significant amount, whereas, according to ref. 14, they are present in the entire column of anaerobic water. In this connection, it is impossible to explain the coincidence of the results for $\sigma_t = 16.50$ and a significant difference between them for $\sigma_t = 16.30$.
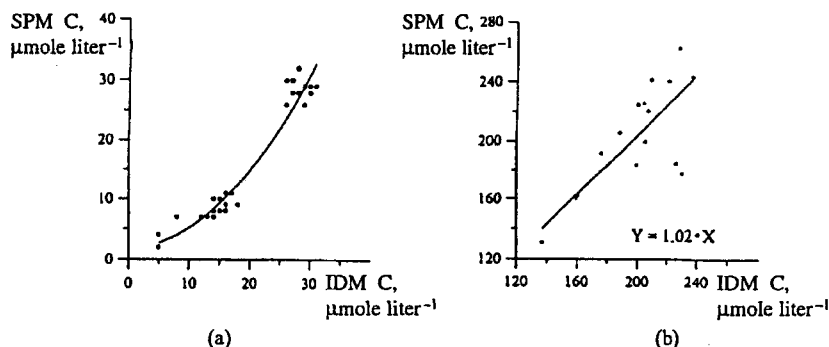

Figure 3. Comparison of the results of parallel detection of hydrogen sulfide by the spectrophotometric and iodometric methods in the range of concentrations 0–40 µmole liter^{-1} (a) and 120–280 µmole liter^{-1} (b).

In the analysis of samples with hydrogen-sulfide concentrations in the range 0–40 µmole liter^{-1}, which corresponds to the upper part of anaerobic zone, we obtained the following results on parallel detection by two methods (Fig. 3a): At the level of relatively low concentrations (below ~30 µmole liter^{-1}), the spectrophotometric method led to understated results as compared with the iodometric method, whereas, for hydrogen-sulfide concentrations of ~30 µmole liter^{-1}, both methods gave close results. The overall character of correspondence of the data of two methods is described by a polynomial curve (Fig. 3a).

The results of parallel detection of hydrogen sulfide in samples by the spectrophotometric and iodometric methods in the range of concentrations 130–260 µmole liter^{-1} are presented in Fig. 3b. One can see that, on average, the values of hydrogen-sulfide concentration obtained by different methods coincide to within 2%. However, the results of analysis of individual samples can differ by 10–30%; in this case, any of these two methods can lead to overstated results.

The data presented in Fig. 4 show that both methods are characterized by a substantial spread in the values of hydrogen-sulfide concentration for any individual value of the conventional density; note that, in the iodometric analysis of samples of seawater with minimal content of hydrogen sulfide (below 3 µmole liter^{-1}), the absolute and relative values of spread are larger than for the spectrophotometric method. Thus, the application of the spectrophotometric method is reasonable only in the analysis of samples with small content of sulfides (below 10–30 µmole liter^{-1}). For

higher contents, the use of the spectrophotometric method does not allow one to make a considerable progress in the investigation of the vertical and spatial distributions of hydrogen sulfide in the Black-Sea waters.
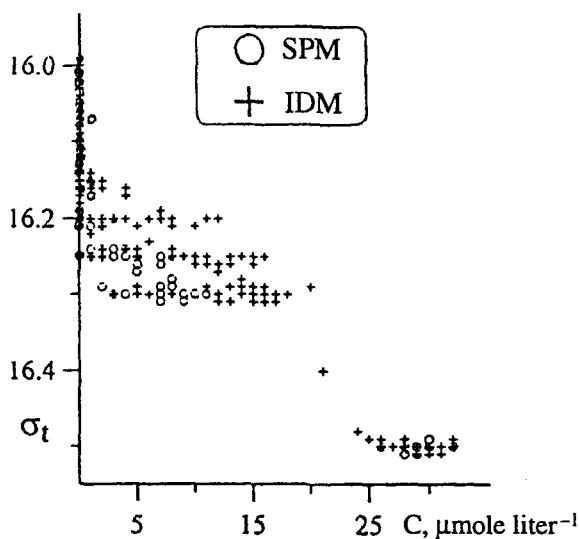

Figure 4. Distribution of the results of spectrophotometric and iodometric analyses of hydrogen sulfide in the Black-Sea waters versus the conventional density.
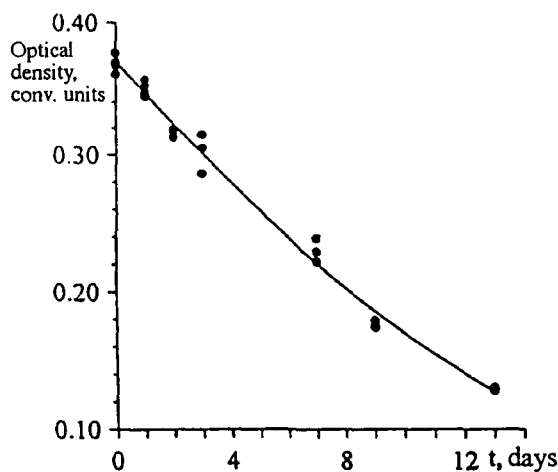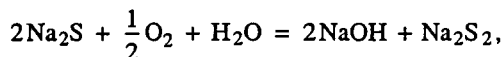

Figure 5. Dependence of the optical density on the time of storage of the main solution of sodium sulfide according to the data of spectrophotometric detection in a sample with dilution 1:200.
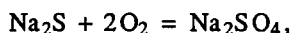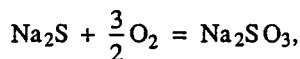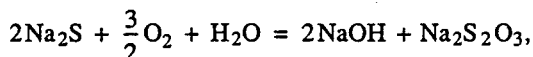
The reasons for significant spread in the spectrophotometric results for individual values of conventional density, which, especially in the case of high content of sulfides, exceed the reproducibility of the method, are unknown. One of possible reasons may be the influence of the products of oxidation of hydrogen sulfide or other substances present in seawater. If such an interfering influence is present, the results of spectrophotometric analysis of samples with low content of sulfides become as ambiguous as those for the iodometric method. The data presented below allow one to look anew at some peculiarities of the spectrophotometric detection of hydrogen sulfide in seawater.

Stability of the standard solution of sodium sulfide. During the 10-day storage of the main standard solution of sodium sulfide in a dark glass-stoppered bottle at a temperature in the range 25–28°C, the optical density in the case of spectrophotometric detection of hydrogen sulfide in a solution with dilution 1:200 decreased from 0.40 to 0.17, i.e., by a factor greater than two (see Fig. 5). In this case, the sum of all reduced compounds of sulfur detected by the iodometric method in a solution with dilution 1:20 remained at the initial level to within 4%.

From the viewpoint of practical application of the spectrophotometric method, this fact indicates that the calibration reference scale must be prepared with the use of a fresh main standard solution of sodium sulfide. The question about the reasons of such a significant difference in the dynamics of the results of spectrophotometric and iodometric analyses seems to be more interesting. If one assumes that the spectrophotometric method is indeed selective and free of the influence of the products of oxidation of hydrogen sulfide on the final results and determines the true content of hydrogen sulfide (H_2S) and the products of its hydrolysis (HS^-, S^{2-}) in samples of water, as stated in ref. 11, then the observed drop in optical density can be explained by the rapid oxidation of sodium sulfide in the main solution. However, it is impossible to propose a scheme of oxidation of sodium sulfide according to which its content decreases by a factor greater than two, whereas the oxidation-reduction equivalent of the solution changes at most by 4%. Even the minimally possible (from the viewpoint of decrease in the oxidation-reduction equivalent of the solution) oxidation of sodium sulfide according to the equation

$$2Na_2S + \frac{1}{2}O_2 + H_2O = 2NaOH + Na_2S_2,$$

and, all the more, according to the equation

$$2Na_2S + \frac{3}{2}O_2 + H_2O = 2NaOH + Na_2S_2O_3,$$

$$Na_2S + \frac{3}{2}O_2 = Na_2SO_3,$$

$$Na_2S + 2O_2 = Na_2SO_4,$$

should lead not only to a decrease in the sulfide content (and, hence, in optical density in the course of spectrophotometric detection) but also to a decrease in the sum of reduced compounds of sulfur determined by the iodometric method and expressed via the equivalent amount of hydrogen sulfide. The assumption that the products of oxidation of hydrogen sulfide affect the intensity of developing coloring when the spectrophotometric method of analysis is used appears more probable. However, in this case, the results of analysis of samples of seawater become ambiguous because optical density would depend not only on the concentration of sulfides, but also on the content of the intermediate products of oxidation of hydrogen sulfide. The data presented in the next section can serve as a confirmation of this statement.

Effect of the products of oxidation of hydrogen sulfide on the results obtained by the spectrophotometric method. In ref. 11, it was stated that the presence of thiosulfates ($S_2O_3^{2-}$) and sulfites (SO_3^{2-}) with concentration up to 30 μmole liter^{-1} in a sulfide-containing sample under analysis does not affect the level of optical density of developing coloring. However, the experiments carried out show that this is not true. Thus, in the analysis of a mixture containing sodium sulfide with concentration 30 μmole liter^{-1} and the same amount of thiosulfate, optical density was lower than in the analysis of pure sodium thiosulfate by a factor of 1.37.

Thus, there exists a quite faithful influence of thiosulfates on the level of optical density of colored solutions. It is quite possible that other reduced compounds of sulfur can also significantly affect the results of spectrophotometric analysis. In this case, the results concerning the indicated (see Fig. 5) drop in optical density during the storage of the main solution of sodium sulfide and in the course of the analysis of the working solution with dilution 1:200 are logically explained by the appearance and accumulation of the products of oxidation of sulfide, which, even in negligible quantities, can significantly affect the results of spectrophotometric detection.

Admitting the presence of the intermediate products of oxidation of hydrogen sulfide in the upper part of the anaerobic zone and taking into account the evidence of their influence on the results of spectrophotometric analysis of sulfides, one can conclude that the spectrophotometric method does not allow one to obtain more reliable information about the content of hydrogen sulfide in the anaerobic zone of the Black Sea. Thus, while the iodometric analysis leads to results overstated by the value of the equivalent content of the compounds capable of reacting with iodine, the spectrophotometric method, with regard at least for the interfering influence of thiosulfates, gives understated data.

CONCLUSIONS

The results of analysis of data presented in literature and performed laboratory and field investigations allowed us to establish the following:

As compared with the iodometric method, the spectrophotometric method is characterized by higher reproducibility of the results of parallel detection only for the content of sulfides in a sample less than 10 μmole liter^{-1} (see Table 2) and by smaller spread in the data of analysis of seawater samples taken in various regions of the Black Sea at depths of isopycnic surfaces in the range 16.10–16.50 (see Fig. 4).

The data obtained by the spectrophotometric and iodometric methods coincide in the mean for the content of sulfides in a sample greater than ~ 30 μmole liter^{-1} (see Fig. 3b), although the results of individual detections may differ by 10–30%.

For the content of sulfides in a sample less than ~ 30 μmole liter^{-1}, the spectrophotometric method gives lower average results than the iodometric analysis (see Figs 3a and 4), which should be taken into account in comparing the results of field investigations carried out by different methods.

The spectrophotometric method is characterized by the presence of interfering influence of the reduced compounds of sulfur, at least of thiosulfate $\left(S_2O_3^{2-}\right)$, on the results of detection of sulfides; therefore, despite the higher reproducibility of results for small contents of hydrogen sulfide, the spectrophotometric method does not allow one to obtain more reliable information about the hydrochemical structure of the anaerobic zone of the Black Sea.

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