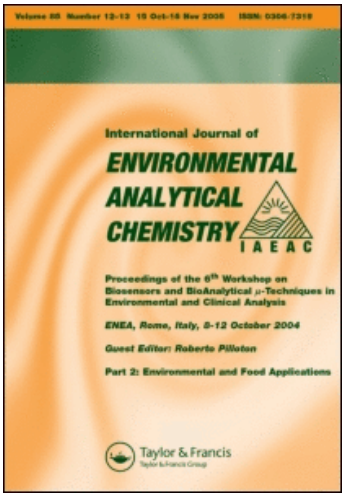

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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To cite this Article Shekhovtsova, Tatyana N. , Muginova, Suetlana V. and Dolmanova, Inga F.(1998) 'Enzymatic Method for Determination of Organomercury in Sea Water', International Journal of Environmental Analytical Chemistry, 69: 2, 191 — 205
To link to this Article: DOI: 10.1080/03067319808032585
URL: http://dx.doi.org/10.1080/03067319808032585

Intern. J. Environ. Anal. Chem., Vol. 69 (2) pp. 191–205
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ENZYMATIC METHOD FOR DETERMINATION OF ORGANOMERCURY IN SEA WATER

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(Received 20 May 1997; In final form 2 December 1997)

The enzymatic determination of organomercury compounds (methyl-, ethyl-, phenylmercury) is based on their effect on the induction period (τ_{ind}) caused by the introduction of sodium diethyldithiocarbamate to the oxidation of o-dianisidine, o-phenylenediamine and 3, 3′, 5, 5′-tetramethylbenzidine by H_2O_2 catalysed by native horseradish peroxidase. τ_{ind} is inversely proportional to organomercury compounds concentration over a range of 0.05-10 μM. The lowest detection limit (C_{min}) is 0.03 μM and the standard relative deviation (RSD) is lower than 3%. The proposed method is simple, inexpensive and does not require the preliminary conversion of organomercury compounds to elemental or ionic mercury. The developed procedure is applied successfully to methylmercury determination in water of Kara Sea.

Keywords: Organomercury; horseradish peroxidase; enzymatic method

INTRODUCTION

It is well known that mercury exists in natural waters in different forms. Organomercury compounds (OMCs) are among them the most toxic ones. Determination of trace quantities of OMCs, and methylmercury especially, in various environmental samples is an important problem of chemical analysis.

Numerous methods for determination of OMCs have been described in the literature[1-21]. They may be divided into two groups. The first group is based on preliminary reduction of OMCs (or their derivatives) to elemental mercury by

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different reducing agents ($SnCl_2$[1, 2], dimethylaminoborane[1], gidrazineborane[1], $K_2S_2O_8$[3], tetraethylborate[3], hydroxilamine[4]) or UV-lamp[5] with subsequent determination by various instrumental methods. The methods of atomic absorption spectroscopy (AAS) with cool vapour[1, 3] and atomic fluorescence (AFS)[2, 4, 5] are widely used for this purpose. The main disadvantage of such methods is the impossibility of determining selectively one particular OMC in the presence of others and Hg^{+2}.

The second group of methods overcomes this trouble. The separation step precedes the determination step. Separation of OMCs is carried out using various types of chromatography (GC[6-7], GLC[8], HPLC[9-11]). A wide assortment of detectors is used for determination of OMCs separated chromatographycally: AAS (variant of cool vapour)[6, 7, 9], electron capture detector[8], ultraviolet[10, 11], electrochemical[12], inductive coupled plasma atomic emission spectroscopy (ICP AES)[13, 14] and mass-spectrometry with ICP[15, 16]. The concentration and separation of OMCs using different inorganic and organic complexing sorbents with sulphur and nitrogencontaning groups[17-19] are applied more rarely. Solutions of cysteine and thiourea in HCl are used as eluents. The final determination of mercury is carried out by AAS[17, 18] or X-ray fluorescense methods[19].

Simple, rapid, comparably inexpensive and highly sensitive enzymatic methods are rarely used for determination of OMCs[20, 21]. Those methods are based on an inhibition of urease with OMCs in the reaction of alkaline hydrolysis of urea. The rate of this enzymatic reaction was monitored fluorimetrically[20] and potentiometrically[21]. The developed enzymatic methods allowed to determine OMCs at a level of their maximum permissible concentrations in natural waters (0.1-1 nM).

An effect of OMCs on horseradish peroxidase (HRP) was not studied up to now. Earlier, on the basis of the inhibitory effect of mercury(II) on HRP activity in the oxidation of o-dianisidine[22], o-phenylenediamine[23] and 3, 3′, 5, 5′-tetramethylbenzidine (TMB)[23], the most sensitive among the reported methods for mercury(II) determination has been developed. C_{min} were 50 pM, 4 pM and 1.5 pM, respectively. The developed method was used successfully for mercury(II) determination in river and sea waters with different mineral salt contents[23]. Such high sensitivity became possible due to the presence of the sulphur-containing organic compound - thiourea in the indicator systems.

The aim of this investigation was to study the effect of OMCs (methyl-, ethyl- and phenylmercury) on the oxidation of o-dianisidine, o-phenylenediamine and TMB, catalysed by HRP, and to develop enzymatic procedures for the determination of OMCs in samples of Sea water.

EXPERIMENTAL

Reagents

Solid preparations of HRP (EN 1.11.1.7; Reanal, Budapest, Hungary) with RZ = A_{403}/A_{237} = 3.28 were used. Solutions with the enzyme concentration of 5 μM were prepared by dissolving an enzyme preparation in sodium borate buffer (pH 7.0), containing 20% vol. of 0.1 M sodium nitrate solution to maintain a constant ionic strength. The exact concentration of the peroxidase solution was determined spectrophotometrically (403 nm, l = 1 cm, $\epsilon_{403} = 9.4 \cdot 10^4\ M^{-1}\ cm^{-1}$). Solutions with a lower peroxidase concentration were prepared daily with the gradual dilution of the initial solution with sodium borate buffer (pH 7.0). Solid samples and solutions of the enzyme were stored at +4°C.

0.15 M sodium borate (pH 7.0) and 0.1 M potassium hydrogen phthalate (pH 5.0) buffers were used. Solutions of the enzyme substrates (o-dianisidine, o-phenylenediamine, TMB), sodium diethyldithiocarbamate (DEDTC), OMCs (CH_3HgJ, C_2H_5HgBr, C_6H_5HgCl) were prepared daily by dissolving accurately weighed amounts in ethanol; potassium ethylenediaminetetraacetate (EDTA) was dissolved in water.

The preparation of the solution of phenylmercury included the stage of preliminary dissolving an accurately weighed amount of phenylmercury in 3-4 drops of a 0.1 M of HCl. The latter was added in order to decrease the decomposition capability of phenylmercury. Then, the obtained solution was diluted to the required volume by ethanol. All the solutions of OMCs were stored in glass bulbs, kept in 20% HNO_3 for a week, in the dark at +4°C.

The stock solutions of Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+} and Bi^{3+} (1 mM) were prepared by direct weighting of the required amounts of their inorganic salts and subsequent dissolution in water acidified with 1-2 drops of concentrated HNO_3. The stock solutions of Hg^{2+} and Fe^{3+} (1 mM) were prepared by dissolving accurately weighted amounts of metallic mercury and iron (purified by carbonyl method) in 3-4 drops of concentrated HNO_3 and subsequent dilution with water to the required volume.

All chemicals were analytical grade reagents ("Souz Reactive", Moscow, Russia). Doubly distilled, dimineralized water was used throughout.

Samples Preparation

Aquatic samples (1 and 2) containing OMCs were collected from the Kara Sea (Russia) close to the mouths of Enisey and Ob' rivers. The collected samples were acidified with concentrated HNO_3 to pH 4.0, then were transferred to plastic bulbs and stored at 4°C, until analysis.

Instrumentation

The absorbance of the peroxidase solution was measured with a spectrophotometer SF-46 (Russia) (403 nm, l = 1 cm). A photoelectrocolorimeter KFK-2 (Russia) (λ_{eff} 460 and 364 nm; l = 2 cm) was used for measuring the absorbance of reaction solutions. The pH of the buffer solutions was measured by a potentiometer pH-121 (Russia).

The required volumes of solutions of the components of the enzymatic process (namely, peroxidase, OMCs (sample), substrates and H_2O_2) were applied using micropipettes.

Measurement of the Reaction Rate

The rate of all the indicator reactions was monitored spectrophotometrically, because of the formation of coloured products in the oxidation of all the aromatic diamines used as the HRP substrates, and was characterised by the slope ($tg\alpha$) of the kinetic curves constructed as follows: absorbance (A) of reaction solutions at the certain wave length versus time (t). Reactions with an induction period were characterised by two kinetic parameters: values of the mentioned above $tg\alpha$ and the duration of an induction period (τ_{ind}). While determining the OMCs concentration the duration of an induction period was used as an analytical signal.

All experiments were carried out at room temperature. Note that the rate of oxidation of peroxidase substrates changes by up to 2% when the temperature changes by 1°C[24].

The detection limit was calculated according to 3s-criterion for n replicate determinations and at the confidence level (P) 95%.

Procedures

(A) Determination of OMCs in the presence of DEDTC using oxidation of o-dianisidine (I), o-phenylenediamine (II) and TMB (III) (model solutions)

7 ml of 0.1 M potassium hydrogen buffer (pH 5.0), 0.1 ml of 60 nM (I) or 0.2 ml (II) or 0.05 ml of 1 nM (III) peroxidase solution, together with 0.1 ml of 1 mM DEDTC solution in the case of the reactions I and II or 0.04 ml of the same solution in the case of the reaction (III) and 0.01-0.1 ml of a standard OMCs solution over the concentration range of 0.01-1 mM (or distilled water in the case of a blank experiment - in the absence of OMCs) were placed subsequently into a glass test-tube with a ground-glass stopper. Then, 0.1 ml of a 5 mM o-dianisidine solution (I) or 0.1 ml of a 80 mM o-phenylenediamine solution (II)

TABLE I The optimum conditions of peroxidase-catalysed oxidation of o-dianisidine (I), o-phenylenediamine (II) and TMB (III) [23, 25] (0.1 M Potassium hydrogen phthalate buffer solution)

Conditions	I	II	III
pH	5.0	5.0	5.0
λ, nm	460	460	364
C(peroxidase), nM	600	1	10
C (substrate), mM	0.005	0.8	0.03
C (H_2O_2), mM	0.005	1	1
C (DEDTC), μM*	10	10	4

*data obtained in this study

or 0.1 ml of a 3 mM TMB solution (III) and water up to 10-ml volume of the reaction mixture were added into the same test tube. Finally, 0.1 ml of 5 mM (I) or 0.1 M (II, III) H_2O_2 were introduced. It should be noticed that the total volume of the reaction mixture must be equal to 10 ml. At the moment of H_2O_2 adding and mixing the reaction solution, a stop-watch was turned on and the absorbance was measured at 15 s intervals for 4 min at the wave length values indicated in Table I. Kinetic curves were plotted as absorbance vs. time. The duration of the induction period was determined. The calibration graphs for the determination of OMCs were plotted as τ_{ind} to a concentration of an OMC.

(B) Determination of methylmercury in sea water samples using oxidation of TMB

7 ml of 0.1 M potassium hydrogen buffer (pH 5.0) and 0.05 ml of 1 nM peroxidase solution were placed subsequently with 0.5 ml of sample 1 or 1 ml of sample 2 solutions (or 0.5 ml water in the case of blank experiments-in the absence of samples), and 0.3 ml of a 0.1 M EDTA solution, into a glass test-tube with a ground-glass stopper. The mixture was stirred and incubated not less than 30 min. Then, 0.04 ml of a 1 mM DEDTC solution, 1.81 ml or 2.31 ml of water for samples 1 or 2, respectively, and 0.1 ml of a 3 mM TMB solution were added into the same test tube. Finally, 0.1 ml of a 0.1 M H_2O_2 was also added. The total volume of the reaction mixture was equal to 10 ml. At the moment of H_2O_2 adding and mixing the reaction solution a stop-watch was turned on and the absorbance at 364 nm was measured at 15 s intervals for 4 min. Analogous experiments were conducted in the presence of standard solutions of methylmercury (with concentrations range of 0.01-1 mM). The kinetic curves were plotted as absorbance vs. time and the duration of the induction period was determined. The calibration graph for the determination of methylmercury in samples was

plotted as τ_{ind} vs. a concentration of methylmercury. Using this graph the content of methylmercury in the samples was determined.

RESULTS AND DISCUSSION

Initial Considerations

The investigation of the effect of OMCs (methyl-, ethyl-, phenylmercury) on the oxidation of o-dianisidine, o-phenylenediamine and TMB was carried out under the optimum conditions of the indicator reactions stated earlier [23, 25] and presented in Table I. OMCs inhibited the catalytic activity of the enzyme when their concentration was not lower than 10 mM. The nature of OMCs anion had no influence on the rate of all the tested indicator processes.

Earlier[26, 27] it was found that the effect of heavy metal cations such as mercury(II), cadmium(II), lead(II) and bismuth(III) on peroxidase activity in the indicator reactions under consideration changed in the presence of some sulphur-containing organic compounds (SCOC). As thiourea increased significantly the inhibitory effect of mercury(II) the introduction of DEDTC to the indicator processes allowed to differentiate the effect of cadmium(II), bismuth(III) and lead(II)[27]. On the other hand, it was also found that SCOC affected the rate of the indicator processes in different ways so that they could be divided into two groups: inhibitors of peroxidase and its second substrates[27]. Thiourea and its derivatives, included into the first group, decreased the rate of o-dianisidine, o-phenylenediamine and TMB oxidation catalysed by HRP, e.g. inhibited the enzyme. SCOC of the second group, and DEDTC in particular, caused an appearance of an induction period on the kinetic curves of all the reactions mentioned above.

Two-substrate reactions occurring in the presence of DEDTC were characterised by two kinetic parameters: a duration of an induction period (τ_{ind}) and a tgα value corresponding to the processes of individual oxidation of DEDTC and diamines, respectively [26] (Figure 1, curve 2). The appearance of an induction period on the kinetic curves in the presence of DEDTC was explained by competitive reactions of an individual oxidation of DEDTC and diamine in the enzymatic system. As the the capability of DEDTC for oxidation (E = −1.68 mV[27]) is stronger than that of the diamines (for example, E (o-dianisidine) = +0.69 mV[27]), DEDTC oxidizes much easier than diamines. So, until the major portion of DEDTC is oxidised, oxidation of diamine proceeds with a very low rate. The absorbance increases considerably after oxidation of a major quantity of DEDTC, but the rate of diamine oxidation (tgα) in this case is lower than that

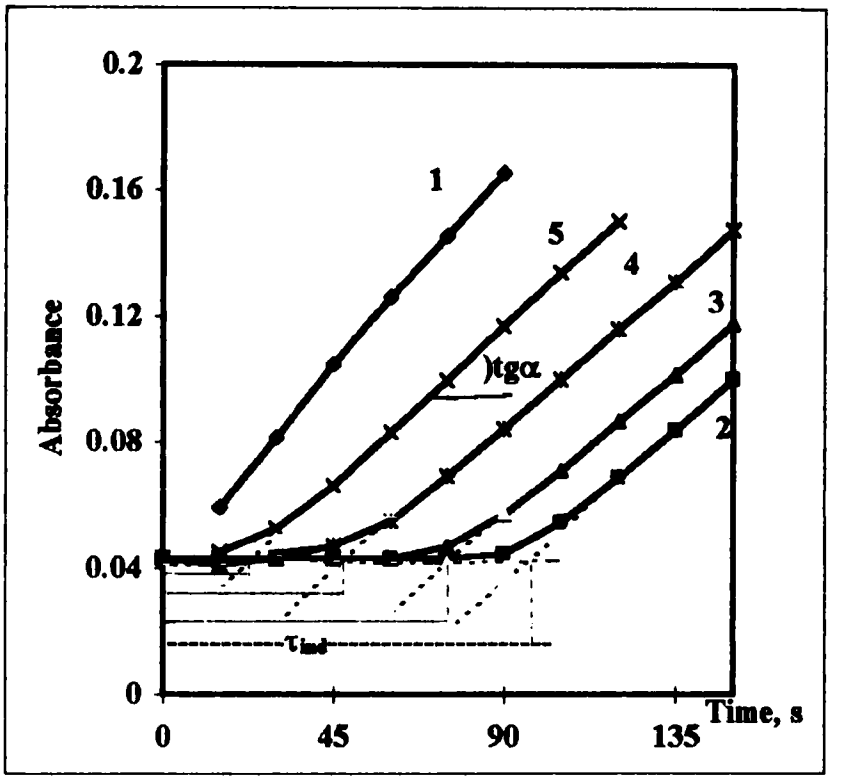

FIGURE 1 Kinetic curves of o-dianisidine oxidation in the absence of DEDTC and methylmercury (1), in the presence of DEDTC (2), in the presence of DEDTC and methylmercury (3-5) (under the optimum conditions of the reaction, concentrations: DEDTC-2-5: 10 μM, methylmercury, μM-3.-0.1; 4.-1; 5-8).

in the absence of DEDTC, because the interaction of DEDTC with an intermediate of diamine oxidation takes place.

Taking into consideration all the data described above we decided to study the effect of OMCs on peroxidase in the reactions of o-dianisidine, o-phenylenediamine and TMB in the presence of thiourea and DEDTC. The results of our investigation showed that thiourea increased insignificantly the inhibitory effect of all the OMCs at concentrations not lower than 10 μM. The combined action of OMCs with substituted thioureas such as acethyl-, allyl- and phenylthiourea caused an increase of the rate of the reaction in comparison with the latter in the absence of OMCs, e.g. the liberative effect of OMCs described earlier [27] was observed.

The most interesting result from the analytical viewpoint was obtained while studying the effect of the OMCs on the oxidation processes of o-dianisidine, o-phenylenediamine and TMB in the presence of DEDTC.

The introduction of the OMCs to the indicator systems decreased the duration of the induction period proportionally to their concentration. At the same time the rate of individual oxidation of o-dianisidine, o-phenylenediamine and TMB was invariable (Figure 1, curves 3 -5). The data obtained showed that the OMCs did not influence the HRP activity in the presence of DEDTC, their effect was related to their interaction with DEDTC, with a complex formation, for example. The possibility of forming a complex of the OMCs with DEDTC was ascertained spectrophotometrically by the example of methylmercury in the oxidation of o-dianisidine in presence of DEDTC (Figure 1, curves 2 and 5). It may be supposed that the duration of the induction period decreases due to a forming complex, which is a less effective reducing agent than DEDTC itself. When the concentration ratio of methylmercury and DEDTC becomes equal to 1, all

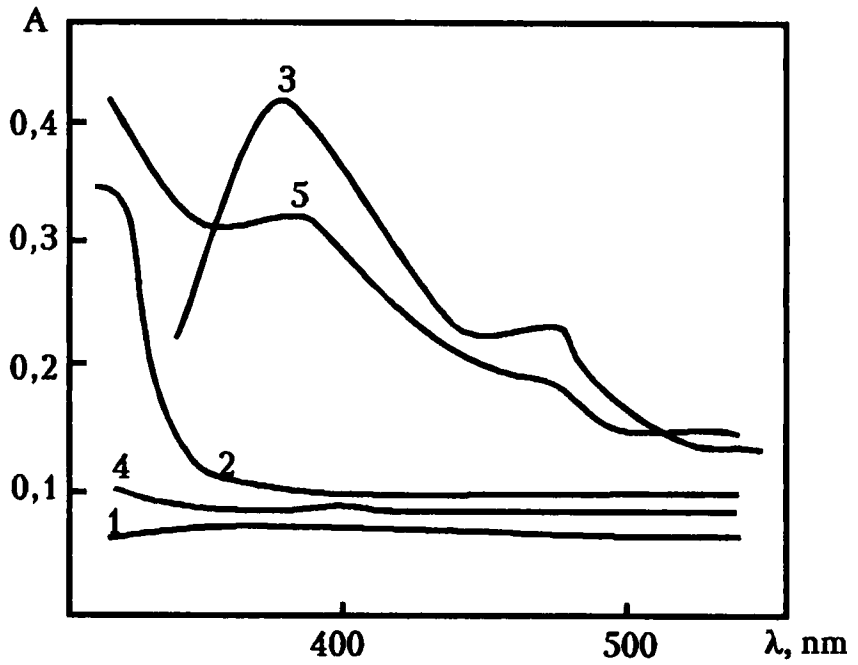

FIGURE 2 Spectra of absorbance for the systems: DEDTC (1), DEDTC-methylmercury (2), o-dianisidine-DEDTC-H_2O_2 (3), o-dianisidine-DEDTC-peroxidase-H_2O_2 (4), o-dianisidine-peroxidase-methylmercury-DEDTC-H_2O_2 (5) (concentrations: peroxidase-60 pM, DEDTC-10 μM, methylmercury-0.2 μM, o-dianisidine, H_2O_2-50 μM, 0.1 M potassium hydrogen phthalate buffer solution, pH 5.0).

TABLE II Analytical characteristics of the procedures for the determination of the OMCs in the presence of DEDTC, using o-dianisidine (I), o-phenelenediamine (II), TMB (III) oxidation (n = 3, P = 0.95)

OMC	Indicator reaction	Applicable concentrations range, μM	Calibration curves equations	r*	C_{min}, μM	RSD, %
CH_3Hg^+	I	0.2-10	y = −8.5 x +90**	0.9996	0.06	3
	II	0.2-10	y = −13.5 x +91	0.9997	0.06	3
	III	0.05-5	y = −8 x +150	0.9996	0.03	3
$C_2H_5Hg^+$	I	0.6-5	y = −7.8 x +90	0.9995	0.4	2
$C_6H_5Hg^+$	I	0.6-5	y = −6.4 x +90	0.9994	0.9	3

*r-regression coefficient, **y = τ_{ind}, s; x = concentration of OMC, μM

DEDTC is bonded into a complex and the induction period on kinetic curves disappears.

The Optimisation of DEDTC Concentration

The optimum conditions for the determination of OMCs studied earlier are presented in the Table I. The enhancement of DEDTC concentration results in an increase of the duration of the induction period. The optimum concentrations of DEDTC (Table I) were judged to be those which caused an appearance of an induction period of 80-150 s. This time was appropriate for the determination of OMCs over the studied concentration ranges.

Calibration

On the basis of the inversely proportional dependence of τ_{ind} of the indicator reaction on the concentration of OMCs in presence of DEDTC, the enzymatic method for their determination was developed. The analytical characteristics of the proposed procedures using o-dianisidine, o-phenylenediamine and TMB oxidation are shown in Table II.

Interference Study

An extensive interference study in view of determining the most toxic methylmercury in natural waters and sea water was performed. The results are summarised in Table III. The selectivity study was carried out using the oxidation of TMB in presence of DEDTC and a fixed concentration of methylmercury (5 μM). A foreign ion was considered not to interfere the

TABLE III Effect of other substances on the determination of 5 μM of methylmercury using TMB oxidation

Foreign ion	MPC[29], μM	Stability constants of complexes with DEDTC [30]	Concentration ratio $C(CH_3Hg^+)$:C(foreign ion) EDTA [31]	$\tau_{ind}-\tau_{ind0}/\tau_{ind0}$*,%		
				before EDTA addition	after 3 mM EDTAaddition	
$C_2H_5Hg^+$	0.001	-	-	1:1	1.5	—
				1:10	4.5	—
				1:100	≥100	—
$C_6H_5Hg^+$	0.001	-	-	1:1	1.3	—
				1:10	4.0	—
				1:100	≥100	—
Hg^{+2}	0.0001	44.56	21.80	100:1	1.5	—
				1000:1	≥100	—
Bi^{+3}	2.5	35.42	27.4	1:1	100	1
Cu^{+2}	1.5	27.01	18.80	1:1	100	1**
Pb^{+2}	0.5	20.80	18.04	1:1	63	2.3
Cd^{+2}	0.05	17.83	16.46	1:1	2.3	—
Zn^{+2}	75	14.01	16.26	1:1	4.5	—
Fe^{+3}	5.5	11.34	24.23	1:2	5	1.9

τ_{ind} and τ_{ind0}-a duration of an induction period in the presence and in the absence of a foreign ion, respectively, average of three determinations, **incubation of EDTA with Cu^{+2} during 30 min, - the literature data are absent, — this step was not necessary, MPC-maximum permissible concentration

determination of 5 μM of methylmercury when caused a change of the duration of the induction period lower than 5%.

There are two types of interferences at least. The first type includes mercury(II), ethyl- and phenylmercury, which often accompany methylmercury in natural samples[28]. It was shown that mercury(II) at the level of its maximum permissible concentrations (MPC) in natural waters of 0.1 ng/mL (0.5 nM) did not interfere with methylmercury determination. The interfering effect of mercury(II) was observed when its concentration was 100 times higher.

The simultaneous presence in the indicator reaction of 5 μM of methylmercury and various contents of ethyl- or phenylmercury resulted in a considerable change of the duration of the induction period (Table III) only at concentration ratios of 1:100 in both cases. Besides, it was found that the combined effect of methyl-, ethyl- and phenylmercury (at 1:1:1 0.1 μM concentration ratio) on the oxidation process of TMB was additive. At the same time, it is well known that a content of methylmercury prevails over that of ethyl- and phenylmercury in aquatic natural samples due to an easy formation of methylmercury under natural conditions and its high stability[32]. We found that if a mixture of OMCs contained methyl-, ethyl- and phenylmercury at a concentration ratio of 8:1:1, its effect on the duration of τ_{ind} became equal to the individual effect of methylmercury. Thus, the developed method for the determination of 0.05 μM methylmercury is selective with respect to the equal concentrations of ethyl- and phenylmercury.

Therefore, as the presence of methylmercury in natural samples (sea-water) seemed to be more obvious than that of ethyl- and phenylmercury the study of the second type of interferences with respect to methylmercury determination was carried out.

The second type of studied foreign ions includes those cations which are able to form stable complexes with DEDTC. The interfering effect was observed for such metal ions as Bi^{+3}, Cu^{+2}, Pb^{+2} which have high stability constants of their complexes with DEDTC (Table III). Fe^{+3} interferes the determination of methylmercury because it is a part of the active centre of peroxidase and enhances the enzyme activity.

The interference of Bi^{+3}, Pb^{+2}, Fe^{+3} at the level of their MPC (column 2 in the Table III) was masked by the addition of 3 mM EDTA. The effect of the most interfering ion Cu^{+2} was eliminated by its preliminary incubation with 3 mM EDTA solution during 30 min. Earlier [25] it was shown that EDTA at the mentioned concentration did not influence the rate of all the considered indicator reactions.

While determining a content of any toxicant, and methylmercury in particular, in sea-water one should take into consideration a content of mineral salts in such

sample. We studied the effect of ionic strength (μ) on the results of the methylmercury determination. The ionic strength was maintained by NaCl and KNO_3 solutions and ranged from 0.05 to 0.5. The data obtained (Figure 3) showed that the effect of methylmercury did not depend on a value of μ over the studied range. The difference of the reaction rate in its absence and presence was constant. On the other hand, it has been stated[27] that the presence of 10% vol. (1 ml in 10 ml of reaction mixture) of water from Black, Caspian, Mediterranean Seas and Sea of Azov (with different extent of salinity) did not influence the results of mercury(II) determination. Therefore, the considered factor (salinity) may be ignored when determining methylmercury in sea water samples.

Analysis of Sea Water Samples

It is known that the contents of methylmercury in open sea waters is much higher than that of ethyl-, phenylmercury and not lower than 50 nM[28, 33]. As it is

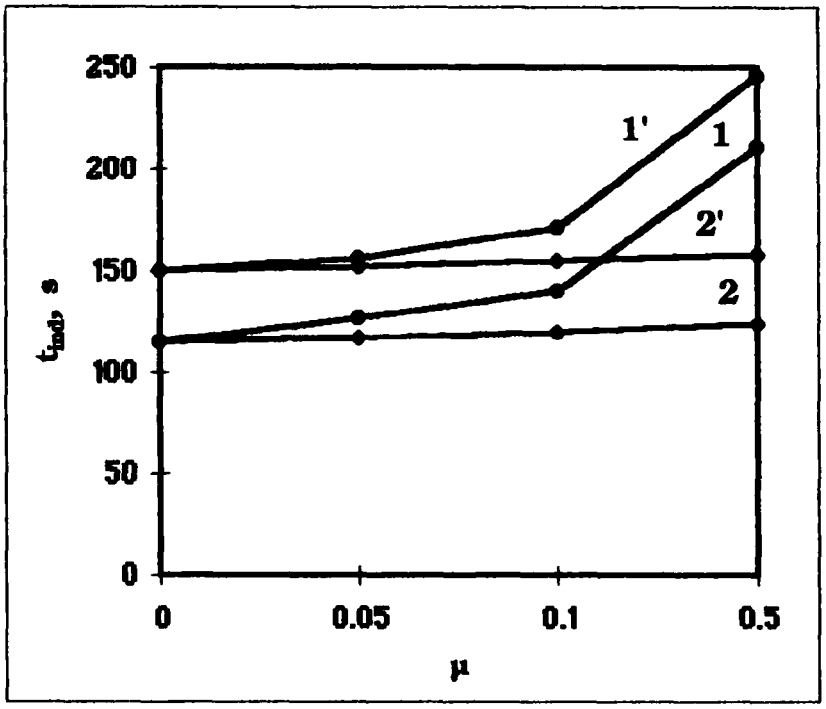

FIGURE 3 Dependence of the duration of the induction period of the oxidation of TMB on the ionic strength created by NaCl (1, 1′) and KNO_3 (2, 2′) solutions in the absence (1, 2) and in the presence of 1 μM of methylmercury (1′, 2′).

TABLE IV Precision and accuracy of methylmercury determination in the tested samples in comparison with another method of analysis

Sample	The proposed method[a]		The alternative method[b]	
	Found	RSD (%)	Found	RSD (%)
1	1.44 ± 0.18	5.1	0.58 ± 0.03	4.7
2	0.60 ± 0.09	7.1	0.51 ± 0.03	5.0

[a]Average of five determinations [b]Average of six determinations

impossible with the developed procedure to determine ethyl- and phenylmercury at concentrations lower than 0.6 μM we assumed that we could determine only methylmercury in the water samples from Kara Sea. For this purpose the procedure of TMB oxidation was used as the most sensitive one. The procedure of the methylmercury determination in the samples (Experimental, Procedure B) was changed slightly in comparison with procedure A, described for the model solutions. So, to eliminate the interfering effect of foreign ions on the methylmercury determination, EDTA solution was added to the mixture of potassium hydrogen phthalate buffer (pH 5.0), HRP and the sample solution. The interfering effect of Cu^{+2} was eliminated incubating the obtained mixture not less than 30 min. The calibration graph (τ_{ind} vs. methylmercury concentration) for the methylmercury determination in the samples was constructed under the optimum conditions presented in the Table I over the range of concentrations 0.05-10 μM. The calibration graph was described by the following regression equation:

$$\tau_{ind} = -7.97[\text{methylmercury}] + 181.52,$$

where τ_{ind} is the duration of the induction period in presence of DEDTC. The regression coefficient is 0.9954 (RSD = 10%, n = 5). The results obtained are shown in Table IV.

The reliability of the developed procedure was demonstrated by the results of interlaboratory studies of the tested samples. The same samples were analysed by the enzymatic method developed earlier[34] and based on the effect of methylmercury on HRP immobilized on polyurethane foams in the TMB oxidation (Table IV). The 3-times difference of the results obtained by both methods in the case of sample 1 may be explained by the fact that the volume of the analysed sample in the case of our procedure is 500-times larger than that in the alternative procedure (500 μl and 1 μl, respectively). So, the summed effect of all possible interferences in the larger probe is stronger and causes higher results.

CONCLUSIONS

Sensitive enzymatic procedures (C_{min} = 0.03-0.9 μM, RSD = 2-3%) for the determination of methyl-, ethyl, and phenylmercury were developed. The procedures are based on the proportional dependence of the duration of the induction period in the process of o-dianisidine, o-phenylendiamine and TMB oxidation catalysed by horseradish peroxidase in the presence of DEDTC. The most sensitive procedure using TMB oxidation was applied for the determination of methylmercury in Kara Sea water. The advantages of the proposed method are its rather high sensitivity, simplicity, rapidity and low cost.

Acknowledgements

This work was partially supported by grant N 95-03-08854 from the Russian Fundamental Research Foundation.

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